Proceedings of The Forum Sessions
Forty-third Clinical Congress of the American College
of Surgeons, Atlantic City, New Jersey
October, 1957

Surgical Forum

VOLUME VIII

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AMERICAN COLLEGE OF SURGEONS
Chicago, Illinois, 1958

LIBRARY OF CONGRESS CATALOG CARD NUMBER 51 2616

PRINTED IN U S A

Foreword

There is no brighter aspect of the American surgical scene than the growing interest in basic research both laboratory and clinical Each year it becomes apparent that facilities for surgical investigation are being steadily expanded that research funds are becoming more adequate and available and that an increasing number of young men are entering the field of surgery both well trained clinically and well grounded in investigative methods There can be no doubt that this fortunate set of circumstances will have far reaching effects not only in extending our fundamental knowledge and in improving surgical practice but also in bringing about better and more inspiring teaching in our schools and hospitals

The American College of Surgeons is proud to have a role in providing the young surgical investigators of this country with a Forum before which their efforts may be presented Year after year it is increasingly evident that the Forum on Fundamental Problems plays a large part in making the annual Congress the outstanding meeting it is The papers presented deal with a variety of important problems in general surgery and in the surgical specialties In this volume these papers are briefly recorded They are carefully written and well illustrated It should be on the bookshelf of every one interested in current progress The College and its Forum Committee are happy to make available this important publication

Again the members of the Committee are deeply indebted to Helene Coleman for editing in an excellent manner the manuscripts included in Volume VIII of the Surgical Forum

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Surgical Forum VIII

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Contents

SHOCK

FLUIDS ELECTROLYTES AND PARENTERAL NUTRITION

WOUND HEALING AND INFECTIONS

TRAUMA, BURNS AND RADIATION INJURY

CANCER

GASTRIC PHYSIOLOGY

LIVER AND BILIARY TRACT

PANCREATITIS

HEART

A – Myocardial Physiology in Heart Surgery

HEART

B — Coronary Physiology, Asystole and Valvular Disorders in the Heart

HEART

C — Problems in the Physiology of Extracorporeal Circulation of the Heart and Vascular Grafts

PULMONARY PHYSIOLOGY, ANESTHESIA AND THYROID GLAND

EXPERIMENTAL HEMORRHAGIC SHOCK: THE EFFECT OF HYPOTHERMIA INDUCED AFTER RAPID ARTERIAL HEMORRHAGE IN THE DOG*

A. THOMAS FERGUSON, JOHN N. WILSON, DALTON JENKINS, AND HENRY SWAN

Beneficial effects of hypothermia on hemorrhagic shock have been reported by many investigators.[1,2,3,4,5,6,7,8] For the last 3 years, this laboratory has been studying a hemorrhage technique consisting of rapid arterial bleeding of 35% of the measured blood volume in splenectomized dogs. This degree of hemorrhage has been shown to be sublethal in the normal anesthetized dog, but is severe enough to cause marked cardiovascular responses. Using this technique,[9] it was demonstrated that hemorrhage in the hypothermic animal was accompanied by a high mortality. This report is concerned with the effect of hypothermia imposed upon the dog immediately after hemorrhage.

METHOD

Splenectomy was performed in order to achieve a more accurate pre-hemorrhage blood volume determination and a more consistent response to a uniform hemorrhage. Twenty-eight adult mongrel dogs were studied. All dogs were subjected to acute arterial hemorrhage of 35% of the measured blood volume.

The dogs were anesthetized with intravenous sodium pentobarbital, 30 mg./kg. Hemorrhage was accomplished by rapid bleeding from the aorta through a polyethylene catheter of 0.106 inches internal diameter inserted upward through the femoral artery. Blood was withdrawn at rates of up to 250 ml./min. The time required to accomplish hemorrhage of 35% of the measured blood volume varied from 3 to 6 minutes.

The blood volumes were measured by the dilution method, using radioactive iodinated human serum albumin (RIHSA). All blood volumes were determined in the warm state. The hypothermic animals, in addition, were given respiratory support during the hypothermic period. No other supportive therapy was given and antibiotics were not employed. Animals which survived 7 days after hemorrhage were classified as survivors.

Hypothermia was established by surface cooling in ice water, as previously reported from this laboratory.[10] Blood pressures were measured directly by means of a femoral artery catheter. A mercury thermometer was used to measure rectal temperatures.

Group I. Ten dogs were anesthetized and after 30 to 90 minutes, (a

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Miss Virginia Beresford performed the careful determination of blood volumes in this study.

period allowed for stabilization of the circulatory system), the blood volumes were determined Rapid arterial hemorrhage of 35% of the measured blood volume was then performed The anesthesia was maintained for 3 hours, after which time the dogs were allowed to recover spontaneously

Group II. Ten dogs were anesthetized, and after stabilization, the blood volume was determined as in Group I The dogs were then cooled to 25°C rectal temperature Rapid arterial hemorrhage of 35% of the blood volume was performed, and after 3 hours, the surviving animals were rewarmed to 35°C by immersion in 40°C water

Group III. Eight dogs were anesthetized, and their blood volumes were determined as in Groups I and II Arterial hemorrhage of 35% of the blood volume was performed, and within 5 minutes following hemorrhage, the animals were immersed in ice water until their rectal temperatures approached 25°C After 3 hours of hypothermia, the animals were rewarmed by immersion in 40°C water

RESULTS

Survival. In the normothermic animals (Group I), no deaths occurred following hemorrhage All animals survived 7 days

When the cooled dogs were bled (Group II), there was an 80% mortality, and one of the two surviving dogs had a bloody diarrhea for several days

Table 1

	NO	SURVIVED	DIED	SURVIVAL
GROUP I	10	10	0	100%
GROUP II	10	2	8	20%
GROUP III	8	8	0	100%

On the other hand no deaths occurred if the dogs were cooled (Group III) following hemorrhage, although a bloody diarreha characterized the first two postoperative days

The animals in Group II which failed to survive died in one of two ways Five dogs died of ventricular fibrillation or arrest within 120 minutes after the hemorrhage The liver and lungs of these dogs were markedly engorged with blood and submucosal hemorrhage of the bowel was found in three The other 3 dogs recovered from cooling and achieved a substantial blood pressure which later fell These dogs died within 12 hours of rewarming Autopsy revealed extensive submucosal hemorrhage and hyperemia of the gastrointestinal tract and the liver and lungs appeared anemic

Blood Pressure. The average mean arterial blood pressures and the average rectal temperatures of the normothermic animals (Group I) are presented in Figure 1 Near the end of the acute hemorrhage was a very rapid and profound drop in blood pressure The mean blood pressure at the lowest point was around 30 mm Hg This was followed immediately

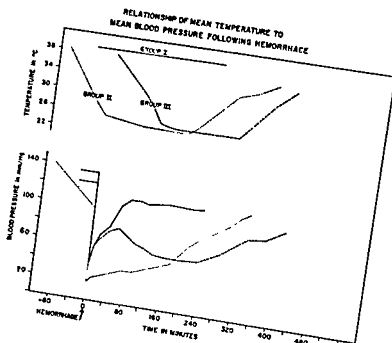


Fig 1

by a rise in the blood pressure which, during the next 20 to 40 minutes, reached a level of 70 to 130 mm. Hg mean pressure.

The average mean arterial blood pressures and the average temperatures of the cooled dogs bled (Group II) are also presented in Figure 1. The blood pressures, which had already fallen with hypothermia, dropped to very low levels (5 to 40 mm. Hg) during hemorrhage. Recovery of the blood pressure, if it occurred at all, was very slow and indeed, with one exception, did not reach normal levels until the animals were warmed. Of these animals, only 2 survived.

The average mean arterial blood pressure and the average temperatures of the bled dogs cooled (Group III) are seen in Figure 1. The blood pressures dropped with hemorrhage and recovered to about 60 to 105 mm. Hg within 50 minutes, much as in Group I, even though these dogs were cooling in ice water during this period. During the next 40 minutes, a second drop in blood pressure occurred as their temperature reached the 32° to 28° range. Upon rewarming, the blood pressures reached levels of 90 to 130 mm. Hg.

DISCUSSION

This study confirms the fact, previously observed in this laboratory, that the splenectomized dog regularly survives an acute arterial hemorrhage of 35% of the measured blood volume, whereas the hypothermic dog, subjected to an equivalent hemorrhage, usually dies. In addition, it demonstrates that the induction of hypothermia following acute arterial hemorrhage is tolerated, although the subsequent 2 days are characterized by a moderate bloody diarrhea. Clearly, *established* hypothermia significantly alters the ability of the dog to compensate for subsequent hemorrhage. However, the results cannot be interpreted as indicating the therapeutic effect hypothermia might have upon hemorrhaged animals. The presence of bloody diarrhea in the dogs which were cooled *following* hemorrhage, Group III, would suggest that the combination was not

entirely innocuous, and that a slightly greater hemorrhagic insult might have reduced the survival rate observed in this group

The manner in which established hypothermia interferes with the dog s capacity to compensate for subsequent hemorrhage is not completely understood The essential difference in the two hypothermic groups (Groups II and III), and directly correlated with survival, is the ability of the dog following hemorrhage to regain and maintain a safe blood pressure The dogs died in the instances where the blood pressure, following its sharp drop with hemorrhage, remained low or continued to drop However, the dogs survived in the instances where the blood pressure began to rise after hemorrhage

Reduced circulating blood volume during hypothermia has been reported[6 11 12] We have not been able to measure to our satisfaction, the blood volume in animals during hypothermia, due to the unpredictability of the concentration curves during the dilution period and the inconsistent extrapolations thus obtained However, there is mounting indirect evidence to the effect that the actively circulating blood volume is indeed reduced during hypothermia[13 14 15 16 17] The most significant indirect evidence is that of Blanco who, using the technique of Walcott,[18] was able to bleed hypothermic dogs only 42% of their warm measured blood volume while in the normothermic dog, the bled volume was 53% of the measured blood volume Our experience in this study substantiates his finding The last portion of the bleeding volume in the cold dog was aspirated with difficulty, as the aorta was completely emptied of blood and collapsed on the catheter For this reason, the total bleeding time in this group was slower The entire bleeding volume in the normothermic dog on the contrary, was always rapidly aspirated without this difficulty

If the circulating blood volume is reduced during hypothermia and as the bleeding volume was based upon the warm blood volume, then the hemorrhage volume employed was much greater than 35% of the circulating blood volume at the time of hemorrhage The circulating volume remaining after hemorrhage in this group would not be comparable with that of the control group until these animals were again warm

SUMMARY AND CONCLUSIONS

1 Three groups of adult splenectomized dogs subjected to acute hemorrhage of 35% of their measured blood volume are presented Group I consists of dogs bled in the normothermic state Group II consists of dogs which were cooled prior to hemorrhage Group III consists of dogs which were bled and then cooled

2 There were no deaths in Groups I and III, while the animals in Group II suffered an 80% mortality Thus, hemorrhage in an already hypothermic dog is usually fatal, while hypothermia imposed on a dog just bled is not fatal

3 There is a direct correlation between the blood pressure responses to hemorrhage and survival A prompt rise in blood pressure following hemorrhage is associated with survival, while a failure or a prolonged delay of the blood pressure to rise is followed by cardiovascular collapse or latent irreversible shock

4 The value of hypothermia as a treatment for hemorrhagic shock is neither confirmed nor refuted, but a noxious effect is suggested by the bloody diarrhea observed in these dogs

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BASIC CONSIDERATIONS OF A METHOD FOR THE CONTINUOUS ELECTRONIC MEASUREMENT OF OPERATIVE BLOOD LOSS*

HARRY H LEVEEN

Operative blood loss has previously been determined by both the hemoglobin and the gravimetric methods Although these methods have enjoyed some degree of common usage, they have definite disadvantages The need for an ideal method led us to investigate the possibility of using electrical conductivity in the determination of operative blood loss

The conductivity method is based on the fact that blood is a solution of almost constant electrolyte composition Even in "low salt syndrome [1] and other more serious disease states [2] the total serum sodium does not vary by more than 10 to 20 mEq The extent of blood loss can therefore be assessed by measuring the quantity of lost electrolyte

Physical Considerations. The electrical resistance of a solution decreases with increasing electrolyte concentration More concentrated solutions of electrolyte will therefore allow for greater passage of current according to Ohm's Law $E = RI$ Conductance is the reciprocal of resistance and is measured in MHOs $MHO = 1/OHM$ The conductance of dilute electrolyte solutions increases in direct proportion to increasing electrolyte concentration Since small changes in conductance can be accurately measured with a Wheatstone Bridge, the change in conductance produced by the addition of blood to a known volume of water could be determined precisely

Individual Variation. The conductivity of diluted hemolized blood of 75 up patients, secured at random, was found to be equivalent to 91 mEq of potassium chloride per liter In 95% of the cases, the variation is less than 3% The greatest variation from the mean was 4 3%

Conductivity of Diluted Blood. If blood is added to distilled water in varying concentrations, a direct proportional relationship between concentration of blood and conductance is found

Effect of Dilution. *If an amount of blood (X) is added to a* known volume of solution (V), the dilution will not be $\frac{X}{V}$ (as is represented in

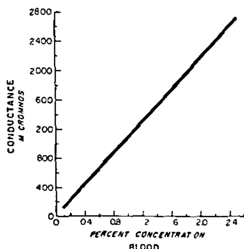


Fig 1 Relationship of conductance to the concentration of blood is illustrated The plot is practically linear

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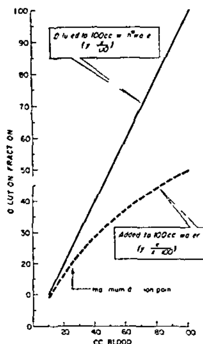


Fig 2 The relationship between the percentile concentration of blood and conductance is illustrated for dilution to 100 cc. of water and for dilution with 100 cc of water The wide divergence at high concentrations of blood is evident In actual practice concentration is kept under 1/4

Fig 1), but $\frac{X}{V+X}$ If an infinite amount of blood is added, the dilution fraction will approach unity The shape of the curve using a volume of 100 cc of aqueous diluent is shown in Figure 2 and it is contrasted with the linear relationship as is shown in Figure 1 It will be seen that the curve deviates sharply only at high concentration In determining blood loss by the conductivity method, it is important that the initial volume of water be great enough so that the final concentration will not exceed 25% blood (This point is indicated by the arrow in Figure 2)

Effect of Protein. Non electrolytes in high enough concentrations will effect a reduction in conductivity The usual concentration of glucose and non protein nitrogen in blood do not produce a perceptible change It is possible, however, that in uremia or non controlled diabetes, values may become sufficiently high to influence conductivity readings slightly Serum protein and hemoglobin do influence conductivity in an ideal way For each gram percent deficit in plasma protein, conductivity increases approximately 2% A blood loss of 100 cc in a patient with a gram percent deficit in plasma protein would appear as a loss of 102 cc This would cause the patient to be overtransfused by 2% A change in hematocrit from 50 to 5 will register approximately 20% increase in loss An anemia of 50% would bring about an over replacement of blood loss by an amount slightly in excess of 10% The method makes moderate corrections for severe hypoproteinemia and anemia

Method of Measurement. Conductivity is best measured on a Wheatstone bridge A conductivity cell is substituted for one resistance in the bridge (Fig 3) Because conductivity increases 2% for each centigrade increase in temperature, a correction must be made for the temperature of the solution This correction is usually accomplished by inserting a calibrated

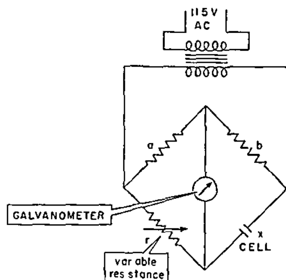


Fig 3 The usual form of a Wheatstone bridge is illustrated No provision is made for temperature correction In the diagram the variable resistance is calibrated and is used to measure the resistance at which nul balance occurs The resistance of the conductivity cell can be calculated from the following

$$x = \frac{b}{a} r$$

variable resistor into one arm of the conductivity bridge Automatic correction can be done by using a thermistor which varies its resistance according to temperature of the solution With a suitable Wheatstone bridge and proper temperature compensation, determinations are accurate to 0 5%

Addition of Automaton. It is possible to have a servomotor bring the Wheatstone bridge into *nul* balance automatically and to have thermistors which make automatic corrections for temperature If an agitator is added blood clots can be quickly and easily extracted of their salt This allows for automatic, instantaneous, and accurate determination of blood loss in the operating room

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PLASMA SEQUESTRATION IN ENDOTOXIN SHOCK*

J BRADLEY AUST, JOHN A JOHNSON, AND M B VISSCHER

Many methods of producing the altered physiological state of "shock" have been described Hemorrhagic neurologic, and shock produced by gram negative endotoxins have all been studied in an attempt to elucidate the underlying factors responsible for the production of irreversibility in these "shock states Fine[3] has suggested that bacterial endotoxins liberated

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from the intestine may play a dominant role in the irreversibleness of hemorrhagic shock states Lillehei[1] and co workers have shown that normally irreversible states of hemorrhagic shock can be tolerated with survival if the intestine is perfused during the period of shock

Recent work in our laboratory by MacLean and co-workers,[2] using gram negative endotoxins, has demonstrated that the gut and liver weight rise during the course of irreversible endotoxin shock Characteristically, the liver weight rises abruptly, associated with an increased portal pressure, then slowly returns to normal The intestinal weight rises gradually over a 4 to 6 hour period until death ensues

It was the purpose of this study to find the nature of the fluid sequestration in such endotoxin shock

METHOD

The kinetics of distribution of I^{131} tagged albumin, CR^{51} tagged red blood cells, sucrose or thiocyanate, and D_2O or antipyrine were studied simultaneously in blood and tissues of normal and endotoxin shocked dogs over a 2 hour period while monitoring arterial, venous, and portal pressures The work to be presented will be concerned with the fate of the I^{131} tagged albumin The differentiation of I^{131} from CR^{51} was made by calculations from the differential decay rates over a 30 day period

The tagged albumin (approximately 100 uc) was injected into normal and endotoxin shocked dogs One cc samples of blood and 1 gm biopsies of skin, muscle, intestine, and liver were taken at 2, 5, 15, 30, 60, and 120 minutes following the injection of tagged albumin These samples were collected in weighed counting tubes, reweighed, and corked These were then counted in a well type scintillation counter Throughout the 2 hour experimental period, arterial pressure, central venous pressure, and portal vein pressures were recorded by Statham strain gauges, and a Sanborn polyviso recorder

The dosage of endotoxin* was one which previous studies had shown to produce irreversible shock in 100% of dogs in 6 to 8 hours Five dogs administered tags alone served as controls for 5 dogs given the same tags following administration of *E coli* endotoxin

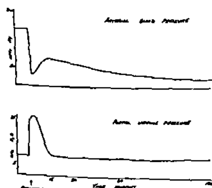


Fig 1

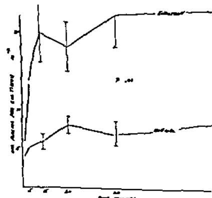


Fig 2

*E coli endotoxin was furnished through the courtesy of Dr Wesley Spink

RESULTS

Figure 1 shows the typical response of blood pressure and portal pressure following the administration of a lethal dose of gram negative endotoxin No changes in these measurements were observed in the 2 hour normal studies Following administration of endotoxin, blood pressure fell abruptly, reaching a minimum at 3 to 5 minutes, at which time the tags were injected Portal pressure rose to a level of from 2 to 4 times normal and remained high for 10 to 15 minutes then returned and remained at pre injection levels Central venous pressure remained relatively constant

Figure 2 compares the distribution kinetics of the I^{131} albumin tag in the intestine of endotoxin shocked dogs with control animals The points indicate mean values and the vertical lines above and below show the standard error of the mean The P values indicate a highly significant difference, consistent with extensive pooling in the intestine Comparison of the distribution of this tag in the liver, muscle, and skin of endotoxin shocked dogs revealed no significant evidence of pooling in these tissues when compared with normal animals

Further studies indicate that this sequestration is limited to the I^{131} albumin, since tagged RBC studies reveal no increase of RBC in these intestines and a marked decrease (over 50%) of intestinal hematocrit following endotoxin administration

DISCUSSION

The finding of plasma sequestration in the intestine of endotoxin shocked dogs corroborates the finding of increased weight of the intestines previously described We believe that this sequestration accounts for the increased weight observed Whether this phenomenon is best explained by plasma skimming, or as an actual loss of protein tags from the vascular tree into the interstitial spaces is open to conjecture If simple pooling and plasma skimming are to account for the observed findings, one would expect an increased amount of RBC in the shocked intestine However, such a finding was not observed, and we therefore favor the concept that capillary permeability has been increased to the point where leakages of large protein molecules into the interstitial spaces can occur

SUMMARY

Dogs subjected to lethal doses of *E coli* endotoxin develop an irreversible shock state characterized by sequestration of a plasma tag in the intestine

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THE EFFECT OF ANTIBIOTICS AND VISCERAL HYPOTHERMIA IN THE PREVENTION OF ISCHEMIC SHOCK*

S. J. LEBRIE, W. M. PARKINS AND H. M. VARS

Fine and his associates[1,2,3] have produced much evidence for a bacterial factor operating in hemorrhagic shock. However, a number of investigators[4,5] have not been able to confirm the effectiveness of antibiotics in preventing death after prolonged hemorrhagic hypotension. Pontius *et al.*[6] reported that control of bacteremia by aureomycin did not change the mortality rate after aortic occlusion.

Edwards *et al.*[7] found that the occlusion of the superior mesenteric and coeliac arteries caused death in 70% of their controls. When pretreated with achromycin only 10% of the dogs died after 90 minutes of occlusion.

Because of these results and the hemorrhagic appearance of the intestine with its sloughing mucosa and the possible breakdown of the barrier to absorption of bacteria or their endotoxins, the following experiments were undertaken. The main purposes were (1) to study the role of a bacterial factor in the genesis of ischemic shock by determining the efficacy of antibiotics in preventing shock and death resulting from aortic occlusion, and (2) to study the usefulness of antibiotics when combined with visceral precooling to 30°C, since precooling alone prevented paraplegia but did not protect all dogs from shock and death after 120 minutes of occlusion. While visceral hypothermia to 25°C initiated immediately after the aorta was occluded protected all animals from death, it did not protect all animals from paraplegia.

METHOD

Healthy adult Beagle hounds were anesthetized with sodium pentobarbital (25 mg./kg.). Both femoral arteries were catheterized, one for blood pressure recordings and the other with a balloon catheter. The latter was positioned so that the balloon was at T 8-10 as described previously.[8]

Hypothermia to 30°C. was induced by a rapid intraperitoneal injection of iced saline (65 cc./kg.). Within 20 to 30 minutes the animal equilibrated both rectal and esophageal temperatures at 30°C. ± 1°C. It was sometimes necessary, when the room temperature was high, to supplement the saline with an iced pack on the abdomen. Rewarming was accomplished by forced warm air heat directed under the animal.

The antibiotics were administered in various doses and time patterns. All dogs receiving neomycin were pretreated for 2 to 3 days.† The antibiotic was administered orally in tablet form twice daily. The total dose was 2 to 3 gm./day depending on the size of the animal. One group of animals receiving neomycin pretreatment was also primed on the day of the experiment, i.e., half of the daily dose was given by gavage with 100 cc. of water 2 hours before occlusion. This gavage was also repeated 3 hours after release of the aortic occlusion.

†Neomycin ("Mycifradin") supplied by the Upjohn Co., Kalamazoo, Michigan.

*From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia. Supported by Army Contract DA-49-007-MD-511.

In the experiments in which a combination of antibiotics was administered, the following were used 2 cc of Combiotics containing 400,000 μ penicillin G and 0 5 gm streptomycin I M, and 15 mg/kg terramycin I V When used as a priming agent the Combiotics were injected immediately after the animal was anesthetized in divided doses between the fore and hind limb Thirty minutes later the terramycin, diluted to 10 cc. with saline, was infused at 1 cc/min via the jugular vein One hour after the Combiotic injection the aorta was occluded

In the post occlusion groups the Combiotics were injected 10 minutes before release of the occlusion, 1 cc into each foreleg The terramycin was injected intravenously at the time the occlusion was released

RESULTS

Sixty Minute Thoracic Aorta Occlusion. Table 1, Group 2, shows the result of 3 day neomycin pretreatment in dogs occluded for 60 minutes Nine of 10 animals survived when pretreated with neomycin Only 40% of the controls survived after 60 minutes of aortic occlusion

Table 1 Antibiotics and Hypothermia in Ischemic Shock Following Temporary Occlusion of the Thoracic Aorta

GROUP NO PROCEDURE	OCCLUSION INTERVAL (MIN)	NO OF DOGS	SURVIVING AT 24 HRS	SURVIVING AT 72 HRS	% SURVIVAL
1 Control	60	10	4	4	40
2 Pretreated Neomycin	60	10	9	9	90
3 Control	90	15	0	0	0
4 Pretreatment + Priming + Post Gavage Neo	90	10	7	6	60
5 Priming Combiotics + Terramycin	90	5	5	3	60
6 Postoccl Combiotics + Terramycin	90	5	0	0	0
7 Precooled 30° C	90	5	5	5	100

Ninety Minute Thoracic Aorta Occlusion. Not one of 15 dogs which served as controls for the 90 minutes occlusion experiments survived, Group 3, Table 1 However, when animals were pretreated for 2 to 3 days with neomycin plus a priming dose by gavage 2 hours before the 90 minute occlusion and another gavage 3 hours after occlusion, 6 of 10 dogs survived over 72 hours Group 4

The animals in Group 5 were primed with Combiotics and terramycin as previously described Sixty per cent of these dogs survived after 90 minutes of aortic occlusion When these same antibiotics, i e Combiotics

and terramycin, were administered at the time the 90 minute occlusion was released, all animals developed irreversible shock and died, Group 6

Group 7 shows the protection afforded by hypothermia When precooled to 30°C all dogs survived 90 minutes of aortic occlusion

One Hundred and Twenty Minute Thoracic Aorta Occlusion. All normothermic control animals died after 120 minutes of aortic occlusion Group 2, Table 2, shows the result of precooling such animals to 30°C Three of 5 dogs, or 60%, survived over 72 hours The same result was obtained when pretreatment with neomycin was combined with the precooling procedure, Group 3 Thus the antibiotics apparently had no additive effect upon the survival rate in these hypothermic dogs

Table 2 Antibiotics Combined with Precooling to 30°C in Ischemic Shock Following Temporary Occlusion of the Thoracic Aorta

GROUP NO PROCEDURE	OCCLUSION INTERVAL (MIN)	NO OF DOGS	SURVIVING AT 24 HRS	72 HRS	% SURVIVAL
1 Control	120	10	0	0	0
2 Control Precooled 30° C	120	5	4	3	60
3 Precooled 30° C Pretreated Neo	120	5	3	3	60
4 Precooled 30° C Priming Combiotics + Terramycin	120	10	7	7	70
5 Precooled 30° C Combiotics + Terramycin Post Occlusion	120	5	4	4	80

Precooling to 30°C, if combined with a prophylactic dose of Combiotics and terramycin resulted in the survival of 7 of 10 dogs When these antibiotics were administered therapeutically at the termination of the occlusion in the precooled animals, 80% survived, Group 5 Thus it would seem that antibiotics do not significantly alter the survival rate in the dog after aortic occlusion of 120 minutes duration under the conditions of our experiments

Hematocrit determinations in all of these groups were of little prognostic significance in predicting the outcome of a particular experiment However, the blood pressure response after release of the occlusion was a fairly accurate index of survival

DISCUSSION

If one makes the assumption that the effect of the antibiotics in these experiments is bacteriostatic or bacteriocidal then one possible explanation for the importance of administration time may be the following When antibiotics are administered before occlusion a reduction in the normal

flora occurs, so that when ischemia is induced the effect of a "bacterial factor" superimposed upon anoxial damage is minimized

SUMMARY

In the 60 and 90 minute occlusion groups prophylactic antibiotic treatment increased the survival rate by 50 and 60% respectively. However, none of the animals survived when the combination of antibiotic therapy was administered at the end of the 90 minute occlusion period

Antibiotics had no significant effect upon survival in the 120 minute hypothermic occlusion groups

In contrast to antibiotics, visceral hypothermia to 25°C initiated immediately after the aorta was occluded protected all animals subjected to 120 minutes of thoracic aorta occlusion

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THERAPEUTIC EFFECTS OF CHLORPROMAZINE IN EXPERIMENTAL HEMORRHAGIC SHOCK*

F G INGLIS, L G HAMPSON, AND F N GURD

The problem of management of the irreversible shock state has recently received attention from investigators interested in hypothermia and autonomic blocking agents[1, 2, 3] These workers observed that animals which were pre treated with autonomic blocking agents, with or without hypothermia, were able to withstand radical surgical trauma as well as traumatic and hemorrhagic shock procedures better than non treated controls The

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foremost of autonomic blocking agents used experimentally was chlorpromazine. It was the purpose of the present investigations to assess experimentally the possibility of using chlorpromazine in the treatment of shock by exhibiting it after the animal had been subjected to the shock procedure.

Due to the large number of variables encountered in a shock experiment, it was decided that the hemorrhagic method would provide the most consistently reproducible form of shock. The only difference between the experimental and the control groups was the administration of divided doses of chlorpromazine after shock had been initiated. No attempt was made to undertake treatment for shock by such methods as repeated blood transfusions, maintenance of fluid and acid-base balance, or antibiotics. Thus it was felt that any differences in results could be attributed to the action of chlorpromazine in the experimental group.

METHOD

Healthy mongrel dogs weighing 15 to 20 kg. were selected at random and premedicated with Demerol one-half hour prior to sodium pentothal anaesthesia. An endotracheal tube was inserted but not inflated. Temperature, pulse, respirations, blood pressure, rate of reabsorbtion from the blood reservoir, duration of hypotension, and volume of blood contained in the reservoir were recorded. Appropriate cut-downs were performed to provide arterial blood pressure recording from the carotid artery and the insertion of a cannula into the femoral artery. From the femoral cannula, the animal was bled into an elevated sterile reservoir where the blood was heparinized. The hemorrhagic shock method of Fine was adopted.[4] There was 100% mortality from this method. In the experimental group divided doses of 2 mg. chlorpromazine were given intravenously every 15 minutes after the animal had reached its maximum bleeding volume and continued until reinfusion of its withdrawn blood. At the conclusion of the experiment, the survival time of the two groups was recorded.

RESULTS

Thirty-three dogs underwent the procedure. Of these, 15 were used as controls and 5 of them died acutely during the experiment. The average survival time of the remaining 10 was 3.2 hours after reinfusion. The experimental group was composed of 18 dogs which received chlorpromazine as indicated above. Again, 5 of these dogs died acutely during the experimental procedure. However, the survival time of the experimental group was significantly prolonged, being 8.02 hours on the average. (See Fig. 1.) Both groups of animals were basically very similar. Average body weights, initial blood pressures, and maximum bleeding volumes practically coincided. The average M.B.V. was 48 cc./kg. for controls and 47 cc./kg. for the experimental group. Rates at which blood was reabsorbed from the reservoir differed somewhat, being 3.72 cc./min. for controls and 4.28 cc. for the experimental group. There was also a difference in the duration of the hypotensive period of the groups. Controls averaged 4.16 hours hypotension, while the experimental group was 3.33 hours at 30 mm. Hg blood pressure. However, a review of the data did not reveal a direct relationship between the period of survival and the duration of hypotension.

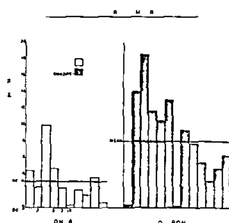


Fig 1 Survival time after hemorrhagic shock. Each vertical column represents the survival time of an individual dog. Means are indicated by the horizontal line. The differences are statistically significant.

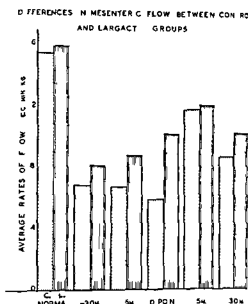


Fig 2 Differences in mesenteric flow between control and largactil (chlorpromazine Poulenc) groups. The vertical column C represents the average value for control flows while the column L represents values for the chlorpromazine treated group. Normal indicates the pre shock values for both groups. Further explanation presented in the text.

There was also no direct relationship between the survival time and the dosage of chlorpromazine beyond 2 mg/kg.

It will be noted that Fine's original method of shock proved to be very lethal and thereby provided no opportunity to study long term survivors. Recently a modification of Fine's method was reported by Postel and others[5] whereby 20% of the controls survived 24 hours. It is hoped to utilize this method in further studies on the shock problem.

A second phase of experimental work was undertaken to determine the influence of chlorpromazine on the flow of blood to the mesenteric vein during shock. The variations in this flow during shock have previously been described by Selkurt and others[6] and their method was utilized in the present experiments. These workers described a progressive fall in the blood flow to the mesenteric vein during the period of hypotension which was followed by a brief return to normal when the animal was reinfused. The hemodynamics of the splanchnic area during shock have been the object of much investigation in recent years. Lillehei recently reported that marked protection against the lethal effects of hemorrhagic hypotension could be obtained if mesenteric artery flow was maintained at normotensive levels during profound hypotension in the peripheral circulation.[7]

The method of measuring mesenteric flow was adapted from the work of Selkurt and others. The animal was prepared as for the previous experiments. In addition splenectomy was performed and the splenic vein cannulized so that flow could be directed through sterile tubing to the

exterior Here the volume of blood flowing per minute was recorded and the blood then reinfused via the femoral vein Blood was diverted to the splenic vein by means of a loose ligature around the portal vein which could be drawn taut for brief periods The animals were shocked according to the method of Fine and in the experimental group divided doses of chlorpromazine were administered intravenously during the period of hypotension Rates of flow were recorded as cc /min /kg

Fifteen dogs were used in these experiments Two of them died during the procedure Five dogs acted as controls while 8 received chlorpromazine as indicated above Results are presented briefly in Figure 2 The zero point represents the average flows just prior to reinfusion Values were then taken at 15 minute and 30 minute intervals before and after the zero point It is evident that a marked increase in blood flowing to the mesenteric vein occurred in those animals receiving chlorpromazine—being 10 25 cc /min /kg while the rate for the controls was only 5 98 cc /min /kg

COMMENTS

As has previously been reported prophylactic administration of chlor promazine has been experimentally effective in protecting the animal against irreversible shock The mode of action of the drug is conjectural but it is generally accepted that by preventing prolonged vasospasm anoxia in vital organs is avoided thereby preventing the onset of irreversible damage to the homeostatic mechanisms The present series of experiments has shown that by administering chlorpromazine to the animal after it had been subjected to conditions which produce irreversible shock a significant prolongation of survival time is obtained

Zweifach and others studied the effects of autonomic blocking drugs on the capillary circulation in the omentum and have shown that these drugs enhance capillary flow during hypotension[8] The results of our present studies concur with this finding for a definite increase in the flow of blood from the superior mesenteric vein occurred following the administra tion of chlorpromazine during hemorrhagic hypotension It will be noted that chlorpromazine has been established as an adrenolytic drug and thereby would appear to have a mode of action opposite to the adrenal medullary group of drugs now receiving widespread use in the treatment of shock This seeming paradox serves to illustrate the current lack in understanding the basic pathophysiology of shock Research in this field should be encouraged and continued As a result of present studies with chlorpromazine it might be reasonable to suggest that investigation be undertaken into the clinical use of the drug in conjunction with blood transfusion when treating cases of severe shock

CONCLUSIONS

1 The administration of small divided doses of chlorpromazine has been shown to prolong survival time in irreversible hemorrhagic shock when given after the animal has reached maximum bleeding volume

2 Small doses of chlorpromazine have been shown to maintain the mesenteric vein flow during experimental hemorrhagic shock

3 That investigation into the clinical use of the drug in conjunction with blood transfusions would be of value in treating cases of severe shock

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THE PHYSIOLOGICAL EFFECTS OF ACUTE ANEMIA PRODUCED BY THE REPLACEMENT OF SERIAL HEMORRHAGES WITH DEXTRAN, PLASMA, AND WHOLE BLOOD*

WARREN WISE, LOUIS R HEAD, MINERVA MORSE, AND J GARROTT ALLEN

When whole blood is not available, dextran or plasma may be used to replace surgical or traumatic hemorrhage Hypovolemia can be corrected, but often at the expense of hemodilution Whole blood must replace the lost red blood cells so as to prevent recurrent shock and the adverse effects of acute anemia The large reserve oxygen carrying capacity of the blood, (two thirds of the total oxygen content[1]), suggests that this portion of the red blood cells might be removed without detriment and that a potentially dangerous blood transfusion might be withheld until this reserve has been partially exhausted

The following experiments were constructed to answer two specific questions (1) What circulatory blood volume can be maintained when blood loss is replaced by plasma volume expanders alone? (2) If the circulatory volume can be maintained, what, if any, are the consequences of the acute anemia that result from the replacement of hemorrhage by these solutions in repeated bleedings?

The comparative effects on hemodynamics, oxygen transport, and the

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buffering system of the blood were studied in 8 adult dogs subjected to serial hemorrhages that were quantitatively replaced by plasma, dextran, or whole blood.

METHOD

Anesthetized, heparinized, adult mongrel dogs were bled one-tenth of their estimated blood volume every 30 minutes until death.[2] Immediately following each hemorrhage, an equal volume of plasma, dextran,* or whole blood was transfused into the femoral vein. Prior to each bleeding episode, venous hemoglobin and hematocrit were determined and the mean arterial blood pressure was established by mercury manometer or a strain gauge attached to a cannula in the femoral artery. Each animal received 100% oxygen through a tracheotomy tube under atmospheric pressure. Oxygen consumption was determined by a standard Benedict-Roth basal metabolism machine. Arterial and venous oxygen saturations were measured by a recording oximeter connected with a Wood type cuvette and by NaHas spectrophotometric analysis.[3] These were spotchecked by Van Slyke analysis. Na, Cl, K, Ca, P, and pH values were determined on venous blood. Cardiac output was calculated by the Fick principle.

*Abbott's Dextran 6% N/S, average molecular weight 40,000.

RESULTS

When whole blood was used as the replacement fluid, the hematocrit reading remained above 40%. With dextran and plasma, terminal levels

Table 1. Mean Arterial Blood Pressure Before and After Multiple Bleedings

DOG NO.	FLUID	BLEEDING NO.	B.P. BEFORE BLEEDING	B.P. AFTER BLEEDING
27	Dextran	1	136	116
		2	126	112
		3	128	108
		4	118	70
		5	94	50
		6	96	46
		7	94	44
		8	94	38
312	Plasma	1	138	92
		2	135	92
		3	135	90
		4	124	90
		5	126	94
		6	114	95
		7	112	94
		8	120	94
355	Blood	1	135	120
		2	130	130
		3	130	130
		4	140	140
		5	160	125
		6	130	130
		7	140	130

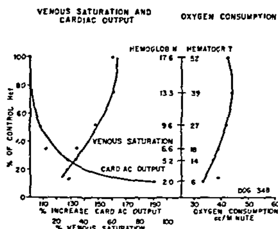


Fig 1

of from 9% to 2% were produced All dogs on dextran or plasma died less than 30 minutes after their last bleeding The onset of death was rapid, once the arterial pressure began to drop, generally within a minute or two

Table 1 shows the changes in mean arterial pressure The response from whole blood replacement indicates the kind of changes that might be expected The animals receiving dextran showed a gradual fall in the mean arterial pressures prior to each bleeding episode and a more radical post bleeding pressure as these episodes progressed than occurred when plasma was given

The dogs receiving plasma and dextran had a 160% to 200% increase in cardiac output during the course of development of hemodilution as bleedings increased in number, especially after the red cell mass fell below 50%

In the animals receiving replacement as either plasma or dextran, the oxygen consumption in all animals was decreased, ranging from 15% to 48% of the 100% control value The decrease averaged 31% This decrease in oxygen consumption was usually preceded by an increase in its consumption, but at the point when the oxygen consumption dropped below control levels a marked increase in cardiac output occurred which was accompanied by a decreasing venous oxygen saturation (See Fig 1) This change occurred when the concentration of hemoglobin reached 6 to 9 gm %, which was about 50% below the starting levels In Table 2 is

Table 2

HEMOGLOBIN	ARTERIAL O_2 CONTENT VOL %	VENOUS O_2 CONTENT VOL %	AMOUNT LOST TO TISSUES VOL %	PER CENT O_2 OF AVAILABLE OXYGEN LOSS TO TISSUE
19.6 Gm (control)	26.62	18.64	8.00	30.7
13.1 Gm Bleeding 4	19.25	8.47	10.78	55.9
5.6 Gm Bleeding 8	9.05	1.85	6.20	77.0

Dog 448

shown the per cent of available oxygen which was extracted from the arterial blood as it passed through the capillaries.

In these animals a terminal acidosis developed with a pH of 6.72 to 7.15 and the plasma CO_2 content declined to one half of its initial level. Deaths occurred from simultaneous arrest of respiratory and cardiac action.

The hemoglobin levels, cardiac output, and O_2 consumption of the animal receiving whole blood replacement by contrast did not change significantly. The pH and oxygen saturations also were not altered.

DISCUSSION

The periods of shock occurring during dextran replacement complicated the interpretation of the effects of anemia in these animals. The changes in venous saturation, oxygen consumption, and minute cardiac output in the plasma dogs appear directly related to the extent of acute anemia. Few changes were apparent before the hemoglobin levels decreased below 50% of the control levels. Once 50% or more of the total circulating hemoglobin has been removed, there is an acceleration of pulse rate, an increase in cardiac output, and an increase in the differences of oxygen saturation for arterial and venous blood.[1] The rise in pulse rate and the increased cardiac output probably represent the usual compensatory mechanisms of acute blood loss. The increased differences between the saturation of arterial and venous blood implies a greater loss of oxygen as blood passes through the capillary circulation. However, despite the greater loss of oxygen saturation as blood traverses the capillary circulation, tissue hypoxia occurs because the extensive hemodilution has produced such a severe anemia that the tissue demands for oxygen are no longer met nor compensated for, once 50% or more of the total circulating hemoglobin has been lost through hemorrhage. Either or both of two mechanisms may account for the increased differences for oxygen saturation of arterial and venous blood. First, under the conditions of these experiments the demand for tissue oxygen may be sufficiently great that the releasing of oxygen from the red cells in the capillaries and its transport to tissues may be increased per gram of hemoglobin involved. Second, despite the evidence that cardiac output is increased as severe anemia develops this does not necessarily imply that some increased stasis in the capillary circulation has not occurred either through partial sedimentation of anemic blood or alterations in blood volume. Either of these mechanisms could cause a delay in passage of the red cells through the capillary circulation and consequently afford more time for oxygen transport to tissues. The pre terminal acidosis appears to be metabolic in origin and to be caused in part at least by the loss of a buffering capacity of the circulating red cells as the acute anemia progresses.[4]

CONCLUSIONS

1 Plasma under these experimental conditions can maintain the venous arterial blood pressure above shock levels surprisingly well until severe hypoxia causes respiratory arrest and circulatory collapse.

2 Dextran used as a replacement fluid in these experiments was less effective than plasma replacement in maintaining the mean arterial blood

pressure, but was equally effective in asserting oxygen transport of anemic blood

3 The compensatory mechanisms for the resulting hemodilution are rapidly exhausted when the hemoglobin falls to levels less than 50% of the control level at which concentration of tissue hypoxia becomes immi nently evident

4 The use of whole blood as a replacement fluid was associated with none of the above changes

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THE EFFECT OF NOREPINEPHRINE ON SURVIVAL IN EXPERIMENTAL ACUTE HEMORRHAGIC HYPOTENSION*

A STEPHEN CLOSE, JOHN A WAGNER, RALPH A KLOEHN, JR, AND ROSS C KORY

Norepinephrine has in recent years received widespread clinical use in the treatment of hypotension *per se*, despite the fact that laboratory studies have indicated that in most circumstances exogenous vasoconstrictors are harmful in hemorrhagic and traumatic shock [4 5 7] These studies, however, *antedated the synthesis of norepinephrine which is the specific active* substance mediating adrenergic neuroeffector cell transmission [1] Norepine phrine has been shown to produce constriction in every system studied with the possible exception of the coronary arteries Gilmore[2] reported an increase in cardiac output following the administration of norepine phrine in all but the terminal stage of shock in dogs bled by the Wiggers technique Morris[3] observed an improved renal blood flow following nore pinephrine administration in hemorrhagic shock Both of these authors recommended that norepinephrine be evaluated clinically in the treatment of hemorrhagic shock during the period of preparation for transfusion Since improvement of cardiac output and renal blood flow do not assure a better prognosis in hemorrhagic shock it seemed desirable to evaluate the effect of norepinephrine on mortality in experimental hemorrhagic shock.

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METHOD

Mongrel dogs weighing from 9.0 to 18.0 kg. were given 1.5 mg./kg. of heparin intravenously, and the left femoral artery and vein were cannulated. A mercury manometer was then connected to a side arm of the arterial cannula. The mean arterial pressure was reduced to 70 to 80 mm. Hg as rapidly as possible (1.5 to 2.5 min.) by arterial hemorrhage. By repeated frequent small bleedings the mean pressure was reduced to 40 mm. Hg exactly 7 minutes after the onset of bleeding. During the succeeding 10 minutes the pressure was maintained at 40 mm. Hg by additional bleeding as necessary. The total volume of blood removed during this 17 minute period is the "Initial Bleeding Volume" (Fig. 1).

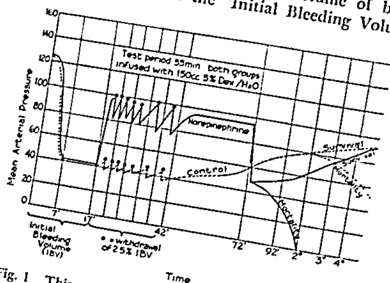


Fig. 1 This graph illustrates the usual responses of dogs in both the control and "treated" groups of Series 1 and 2. Responses of Series 3 dogs were similar.

During the next 25 minutes 17.5% of this "IBV" was removed as follows: 2.5% every 2.5 min. for 12.5 min.; then 2.5% every 5 min. for 10 min. This tedious technique was evolved from a series of experiments in 27 dogs and consistently yielded an average mean pressure of 40 mm. Hg throughout the secondary 25 minute bleeding period.

Series 1: This group of 18 animals was anesthetized with intravenous pentobarbital, 30 mg./kg. Nine of these dogs were used as controls and 9 were "treated" with intravenous norepinephrine solution (12 ug/ml.) throughout the 25 minute secondary bleeding period and for 30 additional minutes. (See Fig. 1.) The flow rate of norepinephrine was adjusted to maintain the mean arterial pressure at approximately 90 mm. Hg. The maximum infusion rate permitted was 36 ug/min. and was not exceeded even though some animals did not maintain a pressure of 90 mm. Hg. This infusion rate is well below that noted to be cardiotoxic in other experiments in our laboratory. The fluid volume containing this maximum norepinephrine dose was 110 ml. In the few animals requiring less than the maximum dose, 5% dextrose solution was given to bring the total infused volume to 110 ml. All control animals received 110 ml. of 5% dextrose solution during the 55 minute test period.

Series 2. The experiments in this series of 37 dogs were carried out as in Series 1 except that morphine analgesia, 2 mg /kg was used instead of pentobarbital anesthesia

Series 3: The 32 animals in this group received pentobarbital as in Series 1 However, in the 16 treated dogs the norepinephrine was not administered until after the completion of the entire 42 minutes of bleeding and the duration of administration was increased to 2 hours The total amount of fluid administered during this 2 hour period was increased to 300 ml in both the control and treated animals to accommodate the longer period of norepinephrine administration

RESULTS

Table 1 summarizes the results in the 3 series of experiments, including mean weight of the animals and the mortality figures

The mortality rate in Series 1 was 33% for the controls and 78% for the animals receiving norepinephrine ($p < 01$) In Series 2 the mortality rate was 35% in the control group and 59% in the norepinephrine group ($p < 05$) In Series 3, 31% of the control animals died and 62% died in the norepinephrine group ($p < 01$) Combining the 3 groups yielded a mortality rate of 33% for the controls and 64% for the norepinephrine group ($p < 01$)

Table 2 compares the mean blood loss (expressed as per cent of body

Table 1

	NO DOGS	MEAN WT IN KILOS	NO DEATHS	% MORTALITY
	SERIES 1 NEMBUTAL ANESTHESIA			
Control	9	12 8	3	33
Levophed	9	12 5	7	78
	SERIES 2 MORPHINE ANALGESIA			
Control	20	12 4	7	35
Levophed	17	10 5	10	59
	SERIES 3 NEMBUTAL ANESTHESIA			
Control	16	11 0	5	31
Levophed	16	12 0	10	62

Table 2

SERIES		MEAN % BODY WEIGHT BLOOD LOSS ALL ANIMALS	SURVIVORS	NON SURVIVORS
1	Control	5.3	5.2	5 7
	Levophed	5 4	5 6	5 4
2	Control	5 7	5 8	5 4
	Levophed	5 9	5 7	6 0
3	Control	5.2	5.2	5 1
	Levophed	5.2	5 1	5 2

weight) in the surviving and nonsurviving animals of the control and treated groups of each series The figures reveal no consistent differences in the percentage blood loss between either the surviving and nonsurviving animals or between the control and norepinephrine groups in any of the series Eighty four per cent of the deaths in the animals receiving norepinephrine occurred during the first 3 hours after completion of bleeding whereas only 50% of the deaths in the control animals occurred during this period When norepinephrine was discontinued, the arterial pressure promptly dropped, and the return to near normal levels in this group was much slower than in the control animals The usual pattern of these changes is shown graphically in Figure 1 in the period indicated between the 72 minute and 4 hour lines

DISCUSSION

Previous investigations have for the most part, supported the concept that an excessive increase in vasoconstriction is harmful during hemorrhage The results of this study support this thesis The mechanism of this harmful effect is not clear Normally the response to rapid hemorrhage includes augmentation of sympathetic nerve activity and endogenous release of pressor amines in physiologically potent amounts This results in severe constriction of the vessels supplying the skin, extremities, salivary glands and spleen [9] However total peripheral resistance may increase only slightly or not at all [6, 8] The observed degree of peripheral constriction without a corresponding increase in total peripheral resistance indicates that the flow pattern between various vascular beds is changed in hemorrhagic hypotension This permits a greater proportion of available blood to perfuse vital organs This selective perfusion is controlled in part by autonomous influences resulting from local metabolic factors, particularly the dilating stimulus of local anoxia Though administered pressor agents may exert a considerable and beneficial constrictor effect peripherally, they may also overcome the local metabolic factors which normally operate to assure adequate blood flow to the splanchnic bed, liver, and to a lesser extent the heart and skeletal muscle This would accelerate the appearance of stagnant anoxia and irreversibility in the very organs in which adequate function is required for survival from hemorrhagic shock

SUMMARY

A method for evaluating adrenergic or adrenolytic agents during hemorrhage is described By use of this method the effect of norepinephrine during and after a standardized trauma was assessed The mortality rate in control animals was found to be 33% as contrasted to a 64% *mortality* rate in the animals receiving norepinephrine

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Fluids, Electrolytes and Parenteral Nutrition

BIOCHEMICAL ALTERATIONS RESULTING FROM VARIOUS INTRAVENOUS REGIMENS GIVEN PRE AND POSTOPERATIVELY I METABOLIC BALANCE STUDIES*

HARVEY KRIEGER, WM E ABBOTT, STANLEY LEVEY, HARRY S SOROFF, ALVIN H HARRIS, AND WM D HOLDEN

Previous studies[1 2 3 4 5] have demonstrated that a significant portion of the metabolic alterations following operative trauma is iatrogenic. A well nourished male patient undergoing a subtotal gastrectomy and maintained postoperatively on an intravenous regimen of hexose and water for 5 days will have an average cumulative 5 day nitrogen deficit of approximately 60 gm When adequate calories and nitrogen are given to other men undergoing a comparable operation the cumulative 5 day nitrogen deficit is reduced to about 8 gm Similarly the postoperative alterations in electrolyte and water metabolism can be influenced by the amount of these substances given The purpose of the present study was to obtain further information, concerning the relative importance of nutrition and operation under better control circumstances, on the postoperative metabolic alterations

METHOD

A group of 10 female patients, aged 20 to 46 years, was studied They had elective cholecystectomies for chronic gallbladder disease but were otherwise healthy The patients were admitted to the Metabolic Division of the University Hospitals of Cleveland, where complete balance studies were conducted Accurate determinations of water, sodium, potassium, and nitrogen intakes and outputs were carried out according to previously described techniques[1] Each study consisted of a 3 day control, a 3 day repletion, and a 3 day postoperative period During the control and postoperative periods the patients were maintained on the same one of three intravenous regimens For 1 or 2 days prior to the control period and during the repletion period a nutritionally complete oral diet was given This was done in order to have the patient in a comparable nutritional state before both the control and postoperative studies The three intravenous regimens which were used were

(1) Five per cent dextrose in water The 3 patients (B J, D W, H W.) maintained on this regimen were given respectively 3000, 3500 and 3250 ml of this solution daily This regimen provided 600 to 700 calories per day and did not contain nitrogen or electrolytes

*From the Department of Surgery Western Reserve University School of Medicine and the University Hospitals of Cleveland This work was supported in part by grants from the National Institutes of Health U S Public Health Service A 760 (C6) the John A Hartford Foundation, Mead Johnson & Company, Evansville, Ind, and Baxter Laboratories Morton Grove, Ill

(2) Five per cent dextrose in water with electrolytes The 3 patients (L B, M O, M J) maintained on this regimen received amounts of 5% dextrose in water comparable to the preceding group and in addition they were given 96 mEq of sodium and 80 mEq of potassium daily, which was distributed in the total volume infused

(3) Five per cent protein hydrolysate, 10% fructose with electrolytes *Each of the 4 patients (S M, E T H R, E H) was given daily 2000 ml* of a 5% protein hydrolysate containing 10% fructose, 92 mEq of sodium and 82 mEq of potassium This regimen was supplemented by volumes of 10% fructose required to make the total fluid and caloric intakes of these 4 patients comparable on a body weight basis The patients were weighed daily and in addition to the aforementioned determinations daily urinary 17 hydroxycorticoids were measured according to the method of *Glenn and Nelson*[6]

RESULTS

Table 1 shows the 3 day cumulative change in nitrogen potassium and sodium during control and postoperative periods for the 10 patients Also shown are the average daily values for the urinary *17 hydroxycorticoids* during the 3 day control and postoperative periods

Nitrogen Balance. The group of patients given adequate nitrogen and

Table 1 Cumulative 3 day nitrogen, sodium, and potassium balances and average daily urinary 17 hydroxycorticoids of 10 patients given 5% dextrose in water, 5% dextrose in water and electrolytes, or a 5% protein hydrolysate containing 10% fructose during both a 3 day control and postoperative period

	NITROGEN (GM)		SODIUM (mEq)		POTASSIUM (mEq)		URINARY 17 HYDROXY CORTICOID (MG)	
5% DEXTROSE	*CONTROL*	*POSTOP*	*CONTROL*	*POSTOP*	*CONTROL*	*POSTOP*	CONTROL	POSTOP
5% DEXTROSE IN WATER								
B J	—16 6	—18	— 89	— 88	—115	—101	4 3	12.5
D W	—20 8	—18.5	— 72	—133	—179	—125	5 3	8 0
H W	—18.2	—21 1	— 41	— 61	—144	—158	4 6	14.2
5% DEXTROSE IN WATER WITH Na AND K								
L. B	—21 1	—33.3	— 76	— 56	— 66	—160	3.9	10.3
M O	—11.2	—15 7	— 43	104	66	— 59	4 7	10.5
M J	— 7.2	—19 4	— 78	— 14	30	— 64		
5% PROTEIN HYDROL. 10% FRUCTOSE WITH Na AND K								
S M	— 8 7	—75	—202	— 56	— 7	— 21	4 8	15 4
E T	— 2 7	— 5 0	— 90	8	34	— 24	4 4	11 4
E. H	0 6	— 0 1	—187	—137	19	— 30	8 4	11 6
H R	— 6 1	— 2 6	—343	—146	64	50	3 8	10 9

calories had 3 day cumulative nitrogen deficits during both the control and postoperative periods which were approximately one fifth that of the other 2 groups of patients who received no nitrogen and minimal calories The patients who received only 5% dextrose in water had nitrogen deficits which were comparable during the control and postoperative periods In some instances the deficit was greater during the control period than during the postoperative period Two of the 3 patients who received electrolytes in addition to the 5% dextrose in water had nitrogen deficits during the postoperative periods which were significantly greater than the nitrogen deficits during their control periods

The patients who were given nitrogen electrolytes and calories had nitrogen deficits during the postoperative periods comparable to the nitrogen deficits during their control periods Three of the 4 patients had greater deficits during the control periods than during the postoperative periods The 6 patients who were maintained postoperatively on a nitrogen free regimen of 600 to 700 calories with or without electrolytes exhibited 3 day cumulative nitrogen deficits ranging between 16 and 33 gm with an average daily deficit of 7 gm On the other hand the 4 patients who received approximately 12 5 gm of nitrogen, electrolytes and about 1500 calories a day had 3 day cumulative nitrogen deficits of 0 1 to 7 5 gm with an average daily deficit of 1 3 gm

Sodium Balance All 10 of the patients had a 3 day cumulative sodium deficit during the control period while 8 of the 10 also had a cumulative deficit during the postoperative period There was no consistent pattern in the sodium balances of the control and postoperative periods In some instances sodium was retained during the repletion period in excess of the deficit of the control period while other patients had a sodium deficit during the postoperative period in spite of cumulative deficits during both the control and repletion periods

Potassium Balance Five of the 7 patients who were given potassium had a positive balance for the control period The other 2 patients and the 3 patients who were not given potassium had cumulative potassium deficits during the control period Nine of the 10 patients had a cumulative negative potassium balance for the 3 day postoperative period while the remaining patient was in positive balance

Urinary 17 hydroxycorticoids The normal range of 24 hour urinary 17 hydroxycorticoids for females according to the method of Glenn and Nelson is 3 5 to 6 0 mg During the control and repletion periods the daily urinary 17 hydroxycorticoids for all the patients were within the normal range A significant increase in the urinary corticoids above the control values occurred during the postoperative period of all patients The maximum increase generally occurred on the day of operation with a gradual return toward normal values over the next 2 days In many instances the urinary 17 hydroxycorticoids were increased by 200% or more during the 3 day postoperative period

DISCUSSION

Until recently there has been a general impression that the metabolic alterations following injury are obligatory because of overactivity of the pituitary adrenal axis and therefore cannot be modified While there is

(2) Five per cent dextrose in water with electrolytes The 3 patients (L B, M O, M J) maintained on this regimen received amounts of 5% dextrose in water comparable to the preceding group and in addition they were given 96 mEq of sodium and 80 mEq of potassium daily, which was distributed in the total volume infused

(3) Five per cent protein hydrolysate, 10% fructose, with electrolytes Each of the 4 patients (S M, E T, H R, E H) was given daily 2000 ml of a 5% protein hydrolysate containing 10% fructose, 92 mEq of sodium, and 82 mEq of potassium This regimen was supplemented by volumes of 10% fructose required to make the total fluid and caloric intakes of these 4 patients comparable on a body weight basis The patients were weighed daily and in addition to the aforementioned determinations daily urinary 17 hydroxycorticoids were measured according to the method of Glenn and Nelson [6]

RESULTS

Table 1 shows the 3 day cumulative change in nitrogen, potassium and sodium during control and postoperative periods for the 10 patients Also shown are the average daily values for the urinary 17 hydroxycorticoids during the 3 day control and postoperative periods

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Table 1 Cumulative 3 day nitrogen, sodium, and potassium balances and average daily urinary 17 hydroxycorticoids of 10 patients given 5% dextrose in water, 5% dextrose in water and electrolytes, or a 5% protein hydrolysate containing 10% fructose during both a 3 day control and postoperative period

	NITROGEN (GM)		SODIUM (mEq)		POTASSIUM (mEq)		URINARY 17 HYDROXY CORTICOID (MG)	
5% DEXTROSE	CONTROL	POSTOP	CONTROL	POSTOP	CONTROL	POSTOP	CONTROL	POSTOP
5% DEXTROSE IN WATER								
B J	—16 6	—18	— 89	— 88	—115	—101	4 3	12.5
D W	—20 8	—18.5	— 72	—133	—179	—125	5.3	8 0
H W	—18.2	—21 1	— 41	— 61	—144	—158	4 6	14.2
5% DEXTROSE IN WATER WITH Na AND K								
L B	—21 1	—33 3	— 76	— 56	— 66	—160	3 9	10.3
M O	—11.2	—15 7	— 43	104	66	— 59	4 7	10.5
M J	— 7.2	—19 4	— 78	— 14	30	— 64	-	-
5% PROTEIN HYDROL, 10% FRUCTOSE WITH Na AND K								
S M	— 8 7	—75	—202	— 56	— 7	— 21	4 8	15 4
E T	— 2 7	— 5 0	— 90	8	34	— 24	4 4	11 4
E H	0 6	— 0 1	—187	—137	19	— 30	8 4	11 6
H R	— 6 1	— 2 6	—343	—146	64	50	3 8	10 9

calories had 3 day cumulative nitrogen deficits during both the control and postoperative periods which were approximately one-fifth that of the other 2 groups of patients who received no nitrogen and minimal calories. The patients who received only 5% dextrose in water had nitrogen deficits which were comparable during the control and postoperative periods. In some instances the deficit was greater during the control period than during the postoperative period. Two of the 3 patients who received electrolytes in addition to the 5% dextrose in water had nitrogen deficits during the postoperative periods which were significantly greater than the nitrogen deficits during their control periods.

The patients who were given nitrogen, electrolytes, and calories had nitrogen deficits during the postoperative periods comparable to the nitrogen deficits during their control periods. Three of the 4 patients had greater deficits during the control periods than during the postoperative periods. The 6 patients who were maintained postoperatively on a nitrogen-free regimen of 600 to 700 calories with or without electrolytes exhibited 3 day cumulative nitrogen deficits ranging between 16 and 33 gm. with an average daily deficit of 7 gm. On the other hand the 4 patients who received approximately 12.5 gm. of nitrogen, electrolytes, and about 1500 calories a day had 3 day cumulative nitrogen deficits of 0.1 to 7.5 gm., with an average daily deficit of 1.3 gm.

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Urinary 17-hydroxycorticoids. The normal range of 24 hour urinary 17-hydroxycorticoids for females according to the method of Glenn and Nelson is 3.5 to 6.0 mg. During the control and repletion periods the daily urinary 17-hydroxycorticoids for all the patients were within the normal range. A significant increase in the urinary corticoids above the control values occurred during the postoperative period of all patients. The maximum increase generally occurred on the day of operation with a gradual return toward normal values over the next 2 days. In many instances the urinary 17-hydroxycorticoids were increased by 200% or more during the 3 day postoperative period.

DISCUSSION

Until recently there has been a general impression that the metabolic alterations following injury are obligatory because of overactivity of the pituitary adrenal axis and therefore cannot be modified. While there is

no question that adrenal overactivity influences the response to injury metabolic balance studies of a large number of patients has led us to the conclusion that the loss of body weight and wastage of nitrogen and potassium via the kidneys after operation can be negated or significantly diminished by providing adequate intakes of nitrogen calories water and electrolytes The present study demonstrated that patients maintained on deficient nutritional regimens had preoperative nitrogen deficits which were comparable to those which developed after operation When adequate caloric and nitrogen intakes were provided the pre and postoperative deficits were significantly less

In contrast to a previous report[7] no correlation could be demonstrated between adrenal activity as measured by urinary 17 hydroxycorticoids and nitrogen excretion Significant increase in urinary corticoids occurred during the postoperative period while the values during the control period were well within the normal range of the method used This postoperative increase occurred irrespective of the nutritional regimen employed The significance of this dissociation is increased by the fact that the 17 hydroxycorticoids are the adrenal steroids which exert the most profound effect on nitrogen metabolism

Other patients who we have studied in a similar fashion had significantly greater postoperative nitrogen deficits than did the patients of this study when complications such as atelectasis or wound infection occurred We have also noted that male patients usually respond to the same magnitude of operative trauma with greater nitrogen deficits and loss of body weight than do female patients

The data on electrolyte and water balance obtained from the 10 patients shows considerable variation from group to group and among patients within a group Postoperatively, the patients who were not given sodium and those who were given nitrogen calories and electrolytes excreted the largest amounts of sodium in the urine It is of interest to note that most of the patients given sodium during the postoperative period developed sodium deficits although it has been generally accepted that postoperative patients retain sodium In several instances this occurred in spite of a pre-existing sodium deficit These studies again demonstrate that the administration of potassium will minimize or negate postoperative potassium deficits

SUMMARY

1 Metabolic studies were carried out on each of 10 female patients who were maintained on the same intravenous regimen for a 3 day control and postoperative period

2 The average daily postoperative nitrogen deficits of the patients who received only 600 to 700 calories were approximately five times greater than those of the patients who had a good nitrogen and caloric intake

3 No correlation could be demonstrated between the nitrogen deficits and urinary 17 hydroxycorticoids

4 Most of the patients who were given sodium had deficits of this ion during the postoperative period Postoperatively potassium deficits were minimized by giving this ion

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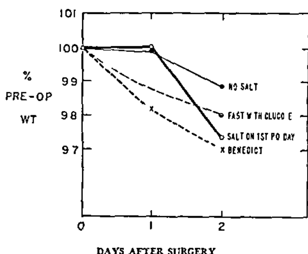
THE PARADOXICAL RELATIONSHIP OF SODIUM CHLORIDE TO WATER BALANCE IN THE EARLY POST-SURGICAL PERIOD*

JAMES H CASEY FREDERICK J NEHER AND BERNARD ZIMMERMANN

The exact amount of sodium chloride to be administered to the surgical patient during the immediate postoperative period is a matter of controversy Despite a considerable amount of investigation since Evans[1] early warning of the dangers of excessive sodium administration to the postoperative patient the whole postsurgical picture of salt and water retention with a resultant tendency toward gain in weight is incompletely understood

In the fasting state the average individual loses about 3% of body weight over a 2 day period[2] while a somewhat lesser weight loss would be anticipated when glucose in water is administered Most surgical patients on the other hand manage to maintain their preoperative weight during the first few days following operation Simultaneously a fall in the serum sodium level generally occurs While water retention and consequent hemodilution are implicated as the responsible mechanism an increase in the extracellular fluid volume has never been consistently demonstrated in the early postsurgical period to account for this[3]

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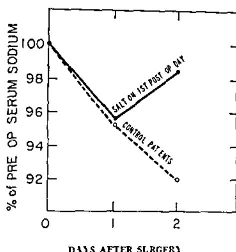


Fig 1 Effect of sodium chloride on body weight when administered on the first postoperative day Control patients received only 5% dextrose in water

Fig 2 Effect of sodium chloride on serum sodium levels when administered on the first postoperative day Control patients received only 5% dextrose in water

Since the advisability of administering sodium chloride during the early postoperative period is debatable, sodium chloride is given somewhat empirically in amounts adequate to prevent symptoms of serious hyponatremia To investigate the effects of various salt loads on the water metabolism and the closely related changes in body weight of the surgical patient, the following experiments were performed

Short term metabolic studies were performed on a total of 37 surgical patients The first group, consisting of 17 patients who had thoracic procedures, received 2500 ml of 5% dextrose in water intravenously daily for the first 48 hours following surgery Ten of the 17 patients also received either 77 or 154 mEq /L of sodium chloride on the first postoperative day while the remaining 7 received no salt While the precipitous fall in serum sodium after surgery in the group receiving no sodium chloride was not unexpected, the difference in weight loss and urine volumes between the two groups was rather striking Figure 1 shows the changes in the average weight curves of the two groups expressed as per cent of preoperative weight For the first 24 hours after surgery both groups maintained their preoperative weight Thereafter the patients receiving sodium chloride 24 hours after surgery lost weight in a manner approximating the course of the nonsurgical fasting subject The average weight loss during the 48 hour period of study in patients receiving sodium chloride was 1 6 kg while in the no salt group only an average of 67 kg was lost †

The changes in serum sodium levels between the two groups expressed as the percent of preoperative serum sodium are shown in Figure 2 Administration of sodium chloride 24 hours following surgery seemed to reverse the downward trend back toward the preoperative level The greatest fall in serum sodium for patients receiving salt was 6 mEq L while it averaged 13 mEq /L in the no salt group ‡ When the salt was administered total

†t = 1 7 n = 15 prob = 11
‡t = 2 9 n = 15 prob = 01

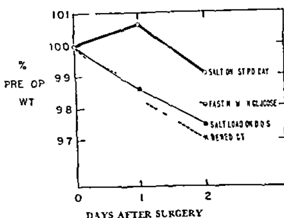


Fig 3 Effect of sodium chloride on body weight when administered on the day of surgery in addition to the first postoperative day Comparison is made with patients receiving sodium only on the first postoperative day

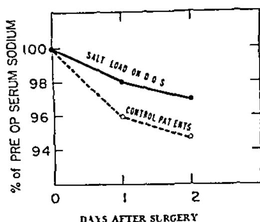


Fig 4 Effect of sodium chloride on serum sodium levels when administered on the day of surgery in addition to the first postoperative day Comparison is made with patients receiving sodium only on the first postoperative day

urine output on the first postoperative day was higher than that of patients maintained on only dextrose in water

In order to find out whether the administration of a sodium load on the day of surgery might further oppose the tendency toward water retention and the fall in serum sodium levels, a group of 20 patients undergoing major abdominal surgery was studied Eleven patients were given 154 mEq of sodium chloride on the day of surgery and 9 received none All received 2000 ml of 5% glucose in water on the day of surgery and 2500 ml of 5% glucose in water with 77 mEq of sodium chloride on the first postoperative day Since the gastric drainage during the study period was nearly equal for both groups, variations in gastric drainage could not account for the observed differences in electrolyte levels or water balance between the two groups

That the group receiving the salt load on the day of surgery was in more physiologic water balance is indicated by the close proximity of its average weight curve to that predicted for non operated fasting subjects on equivalent caloric restriction (Fig 3) On the other hand, body weight was maintained near preoperative levels in the 9 control patients until salt was administered 24 hours after surgery The average weight loss, corrected to the weights of the surgical specimens, during the first 2 days after surgery was 1 75 kg in patients receiving sodium chloride on the day of surgery while it averaged 0 5 kg in the 9 patients without the additional salt load † The average fall in serum sodium again expressed as the percentage of the preoperative level was less in the patients receiving the additional salt load on the day of surgery (Fig 4) The fall in serum sodium over the period of study averaged 4 8 mEq /L in the salt load group as opposed to 8 4 mEq /L in the 9 patients receiving sodium only on the first postoperative day †† The decrease in osmolarity of the serum averaged 7 3 in the salt load group and 13 6 in the control group Urine volumes averaged 1555

†t = 2 6 n = 18 prob = 018
††t = 3 1, n = 18 prob = 006

ml on the first postoperative day with an average sodium content of 65 mEq and an osmolarity of 528 mOsm/L in the 11 patients in the salt load group An average urine output of 1340 ml with a sodium excretion of 21 mEq and an osmolality of 480 mOsm/L was observed in the control group According to the formula Cosm $= \frac{UV}{P}$, an osmotic diuresis was obtained

When a sodium load was given on the day of surgery, weight loss over the next 48 hours averaged 1 25 kg more than for the control group The differences in urine volumes (of slightly more than 200 ml) between the two groups is not large enough to explain this difference Perhaps if a larger series had been used the trend toward greater urine volumes after the administration of the salt load would be more striking On the other hand it is possible that the difference in weight loss between the two groups is due to some unknown source of water loss such as increased perspiration or even an increased loss of water vapor from the lungs secondary to better respiratory excursion as a result of increased well being in the patient experiencing a more physiologic course

DISCUSSION

There is little doubt that sodium depletion and response to hydration are in general closely related Bristol[4] found a definite correlation between serum sodium concentration and the ability of the kidneys to excrete a water load Whereas it is exceedingly difficult to produce water intoxication in normal dogs it is well known that after sodium depletion only a fraction of a forced water load can be readily excreted

Hayes[5] maintains that water should be given slowly and in limited amounts to the surgical patient under the supposition that the kidney of the postsurgical patient is limited by antidiuretic hormone in its ability to excrete urine However the experiments described herein demonstrate even in the immediate postoperative phase that given an osmotic load the kidneys will excrete a large amount of urine, although of relatively high osmolarity Increasing the osmotic load seems to be a more satisfactory approach than relative dehydration in that better renal function may be assumed by the former course

Though increasing serum osmolarity by the administration of other substances such as urea[6] or mannitol[7] can also produce diuresis, the use of moderate amounts of sodium early in the postoperative course would appear to be simpler and perhaps more physiologic

SUMMARY

1 A series of metabolic studies were performed on 37 surgical patients

2 When only 5% dextrose in water was administered, water retention with consequent maintenance of preoperative weight plus a marked fall in serum sodium was observed The administration of a modest amount of sodium on the first preoperative day resulted in a subsequent elevation of serum sodium levels and a change in the weight curve toward that expected in the fasting physiologic state

3 When in addition, sodium chloride was administered on the day of

surgery, the tendency toward maintenance of body weight and toward falling serum sodium levels was further reduced

4 The early administration of sodium appeared to increase both the urine volume and osmolarity

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POSTOPERATIVE SALINE THERAPY*

D MILLAR BELL

During the early post traumatic and postoperative period patients may be neither willing nor able to take an adequate volume of fluid by mouth Furthermore it is often inadvisable to permit them to have fluids by mouth at this stage for example following operations on the gastro-intestinal tract

There is a retention or conservation of sodium by the kidneys for 2 to 4 days following operation and there is a widely held view that it is harmful and unnecessary to give saline during this period It is consid ered that this will result in an overloading with sodium excessive water retention with the production of edema and delay in healing of wounds The delay in healing could be disastrous in intestinal anastomoses

Intravenous infusion was largely introduced in this country by Matas[1] in 1911 using physiologic saline at first but later he gave this up in favor of 5% glucose in water because of the danger of edema following the continued use of physiologic saline

In 1949 Elman and associates[2] made a study of 40 patients to determine the minimum postoperative maintenance requirements of fluid and electro lytes They concluded that the addition of 2 to 4 gm of sodium chloride daily is all that is required

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The purpose of this investigation, therefore, is to determine whether it is detrimental or beneficial to administer physiologic saline during the early postoperative period, as indicated by the mortality in dogs subjected to a severe stress load We were stimulated to perform these experiments because of doubt expressed by Dr John Laws[3] concerning the wisdom of giving no salt in the early postoperative period

METHOD

Fifty two dogs were used divided into 2 groups Group A consisted of 27 dogs, and Group B of 25 dogs

The animals were subjected to two stress loads with an interval of 48 hours between them Stress Load 1 consisted of cholecystectomy and splenectomy through a right upper paramedian incision, left nephrectomy and exposure of the small intestine for 10 minutes through a left para rectus incision This was followed by crushing the hind muscles of one hind limb, using an ordinary hammer with a padded striking surface, 300 blows being delivered Stress Load 2 consisted of celiotomy through a right lower pararectus incision and exposure of the small intestine for 5 minutes This was followed by crushing of the thigh muscles of the second hind limb, 700 blows being delivered

The animals in Group A received 500 cc 5% dextrose in water intra venously immediately following Stress Load 1 Twenty four hours later they received 700 cc, and again after Stress Load 2 In Group B the animals received 500 cc physiologic saline following Stress Load 1 Twenty four hours later they received 500 cc physiologic saline plus 200 cc 5% dextrose in water, and again after Stress Load 2, to make up their daily fluid requirement

Each animal was weighed before each stress load and before infusion on the intervening day A 10 cc blood sample was withdrawn before Stress Loads 1 and 2, and before infusion on the intervening day, from which serum sodium chloride and nonprotein nitrogen values were obtained A group of 10 animals was kept in metabolism cages, urine collected and from aliquots of the samples urinary sodium and chloride values were obtained However, an accurate record of 24 hour urine output was not obtained as the animals almost invariably passed urine when taken out of their cages

Autopsy was performed on those animals which died following Stress Load 2 The survivors were kept under observation for a further 6 days then sacrificed and autopsy performed

RESULTS

Group A (dextrose group). In this group 6 dogs died in 6 to 24 hours following Stress Load 1 a mortality of 22 2% Twelve of the remaining 21 dogs died in 6 to 24 hours following Stress Load 2 a mortality of 57 1%

Group B (saline group) In this group 4 dogs died in 6 to 24 hours after Stress Load 1, a mortality of 16% Six of the remaining 21 dogs died in 6 to 24 hours following Stress Load 2, a mortality of 28 6%

The operative procedures in this experiment produced no unsuspected difficulties Particular attention was given to careful hemostasis Care was also taken to avoid damage to or removal of an adrenal gland The

Table 1 Comparison of Percentage Mortality in Glucose and Saline Groups

GROUP	TOTAL NO OF DOGS	MORTALITY FROM STRESS LOAD 1	MORTALITY FROM STRESS LOADS 1 AND 2
Glucose	27	22.2% (6)	57.1% (12)
Saline	25	16% (4)	28.6% (6)

weights were carefully recorded and it was found that in Group A there was weight loss over the 3 day period ranging from 2.8 kg to 0.1 kg with an average loss of 1.2 kg. In group B there was a gain in weight in three instances over the 3 day period. The weight loss in the others ranged from 1.9 kg to 0.1 kg, so that the average net weight loss was 0.7 kg.

There was very little change in the serum sodium and chloride values in each group.

DISCUSSION

In considering the results in these experiments it should be mentioned that the actual daily requirements of sodium for the dog have not been accurately determined (McCay[4]) but it is considered that on a normal diet 2 to 4 gm represents the daily intake. The dogs in this experiment received about 500 cc of isotonic saline daily, that is 4 to 5 gm of sodium chloride which, based on the average weight, represents 0.3 gm /kg per day. The daily fluid requirement of the dog varies from 500 cc for a small animal to about 1 L for a large animal. The animals in this study, the majority of which were in the intermediate size and weight range, received 700 cc of fluid daily after the first day of operation.

The renal mechanism in the dog with respect to sodium is somewhat different from that in the human. An excess sodium load is excreted more rapidly than is the case with the human being. Davis and Howell[4] have shown that when under stress the dog does retain sodium to a marked degree. There was a well marked retention of sodium following both stress loads.

This investigation was undertaken to evaluate the place of saline therapy in the immediate postoperative period on the concept that improper fluid and electrolyte therapy at this stage would interfere with subsequent cell function, and thus increase either morbidity or mortality. This is in contradistinction to the evaluation of the role of saline in the treatment of shock. It is therefore apparent that for the purpose of this experiment it was necessary to induce a state of stress and likewise to exclude those animals dying after Stress Load 1.

The animals dying in the glucose group can be taken to represent those patients to whom no saline is given postoperatively for 48 hours and the saline group, those who receive saline at this stage.

The mortality from Stress Load 1 was 22.2% in the glucose group and 16% in the saline group. The operative stress load was a heavy one and the slight difference in mortality following Stress Load 1 might be attributable to the value of saline in the treatment of shock. This is open to some doubt because the effect of saline is short lived, 1 to 2 hours, and the effect was not maintained.

Forty eight hours were allowed to elapse between the stress loads so that hemostasis was effected Although the animals were in a state of stress following Stress Load 1, no essential difference could be detected in 24 hours on observation of those in each group Nevertheless when the animals were subjected to Stress Load 2, we found a marked difference in mortality, 57 1% in the glucose group, and only 28 6% in the saline group This would certainly indicate that the animals in the saline group had much better cell function as indicated by their ability to withstand further stress

No difference was detected between the survivors in each group as regards wound healing during the period of observation In no instance was there edema in the wounds or the lungs in the saline group of animals

The postoperative patient loses some sodium in the urine In addition *there is a loss of sodium in sweat (of the human being), which contains* 50 to 100 mEq/L The average daily fluid loss in this way is 800 to 1,000 cc, but much more when there is excessive sweating The combined loss of sodium in the urine and sweat at this stage, will result in a negative sodium balance, in the majority of cases The results of our experiments would indicate it might be desirable, therefore, to give 2 to 4 gm of sodium chloride in each 24 hour period, postoperatively

The danger of administering physiologic saline at this stage is from prolonged or overzealous administration, i e, 2 to 3 L of saline in each 24 hour period for 2 or 3 days postoperatively

SUMMARY

The results of the experiments reported here indicate that the admin istration of isotonic sodium chloride in the early postoperative period is beneficial One group of 27 dogs was subjected to a heavy stress load and received 5% dextrose in water in the postoperative period The mortality was 22 2% Another group of 25 dogs was subjected to the same stress load and received isotonic saline plus 5% dextrose in water in the post operative period The mortality was 16%

Forty eight hours after the first stress operation the survivors (21 dogs in each group) were subjected to a further stress load The mortality in the glucose group was 57 1% and in the saline group 28 6% This might suggest that it is desirable to give saline in the early postoperative period to patients having major operations The beneficial effect of saline in shock is well known and would apply to some extent, but we doubt that this factor exerts a major role in the results

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ALTERATIONS IN BODY COMPOSITION WITH PREPARATION OF CARDIAC PATIENTS FOR SURGERY*

K. H. Olesen, H. V. Parker, and F. D. Moore

A method for multiple simultaneous isotope dilutional studies of total body water and electrolytes, intravascular, extracellular, and intracellular phase volumes has been developed.[1] Characteristic patterns of body composition in certain clinical disease states have been defined, and it will be the purpose of this paper through application of this method to describe the alterations in body composition with preparation of edematous cardiacs for surgery. The multiple information gained in this type of study will expand our knowledge obtained through metabolic balance studies and isolated isotope dilution studies.[2, 3, 4, 5, 6]

METHOD

Four adult males with cardiac edema of short duration were studied during the edematous state and restudied 2 to 5 weeks later after recovery from edema. The treatment given was salt poor diet and digitalis. In 2 patients a few injections of mercuhydrine were used.

Within 48 hours the following measurements were carried out: total body water (using deuterium oxide with an equilibration time of 6 hours), plasma volume (using Evans Blue), red cell volume (using Cr^{51}), blood volume (the sum of plasma volume and red cell volume), total exchangeable chloride (using Br^{82}), total exchangeable sodium (using Na^{24}), and total exchangeable potassium (using K^{42}). The extracellular water was calculated from the volume of distribution of radiobromide, corrected for erythrocyte chloride, plasma water and Donnan effect. The difference between total body water and extracellular water represented the intracellular water.

RESULTS

The results of the measurements are listed in tables 1 to 3. For the sake of brevity the average values of the four studies are given. The measurements are expressed as absolute values, as values relative to the non-edematous weight at the time of the second study, and as percentages of normal compared to the normal values for adult males used in the laboratory.

The serum electrolyte concentrations were within normal limits in all studies.

Body composition during the edematous phase. The results of the measurements during the edematous phase are given in Table 1.

Related to the non-edematous weight the findings were: the total body water represented 62.3% of body weight or 115% of normal. The plasma volume was increased to 4.6% of body weight or 107% of normal, the red cell volume amounted to 3.2% of body weight or 118% of normal, and the total blood volume represented 7.8% of body weight or 111% of normal. The extracellular water volume was markedly increased, represent-

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Table 1 Average Values for Body Composition in Four Edematous C

MEASUREMENT	ABSOLUTE	RELATIVE % BODY WT OR mEq/kg NON EDEM WT	% N NO
Weight	82.5 kg		
Total body water	46 3 L	62 3%	
Intravascular phase			
Plasma volume*	3375 ml	4 6%	
Red cell volume	2412 ml	3.2%	
Blood volume*	5705 ml	7 8%	
Extracellular phase			
Extracellular water	25 7 L	34 6%	
Total exchangeable sodium	4281 mEq	57 7 mEq/kg	
Total exchangeable chloride	3029 mEq	40 7 mEq/kg	
Intracellular phase			
Intracellular water	*20 6 L*	*27 7%*	
Total exchangeable potassium	3062 mEq	41 3 mEq/kg	

*3 patients only

Table 2 Average Values for Body Composition in Four Cardi After Recovery from Edema

MEASUREMENT	ABSOLUTE	RELATIVE % BODY WT OR mEq/kg NON EDEM WT	% A NO W
Weight	74 2 kg		
Total body water	41 1 L	55 4%	1
Intravascular phase			
Plasma volume*	3080 ml	4 2%	9
Red cell volume	2215 ml	3 0%	1
Blood volume*	5209 ml	7.2%	1
Extracellular phase			
Extracellular water	19.5 L	26.2%	1
Total exchangeable sodium	3313 mEq	44 6 mEq/kg	11
Total exchangeable chloride	2367 mEq	31 9 mEq/kg	11
Intracellular phase			
Intracellular water	21 6 L	29 1%	9
Total exchangeable potassium	2918 mEq	39 7 mEq/kg	8

*3 patients only

ing 31.6% of body weight or 150% of normal. The total exchangeable sodium and total exchangeable chloride showed a proportionate increase above normal. The intracellular water volume was slightly decreased, representing 89% of normal, and the total exchangeable potassium showed a proportionate decrease to 88% of normal.

These patients showed a pattern of body composition which is characteristic in cardiac edema: increased total body water, increased plasma volume, red cell volume and total blood volume, markedly increased extracellular water, total exchangeable sodium and total exchangeable chloride, and decreased intracellular water and total exchangeable potassium.

Body composition after recovery from edema. The results of the measurements are given in Table 2.

Related to the non-edematous weight the findings were: the total body water represented 103% of normal. The plasma volume and the red cell volume were close to normal. The extracellular water had decreased to 114% of normal, and the total exchangeable sodium and total exchangeable chloride showed a proportionate decrease towards normal. The intracellular water represented 94% of normal, and the total exchangeable potassium represented 84% of normal.

This pattern of body composition: normal total body water, slightly increased red cell volume, slightly increased extracellular water volume, total exchangeable sodium and chloride and decreased intracellular water and total exchangeable potassium is a frequent finding in non-edematous cardiac patients.

Alterations during recovery from edema. The results are given in Table 3.

The main alteration in the body composition in our series was a reduction in extracellular water of 6.2 L or 36% of normal, a proportionate decrease

Table 3. Average Changes in Body Composition in Four Cardiacs During Recovery from Edema

MEASUREMENT	ABSOLUTE	% NORMAL NON-EDEM. WT.
Weight	—8.3 kg	
Total body water	—5.2 L	—12%
Intravascular phase		
Plasma volume*	—295 ml	—9%
Red cell volume	—197 ml	—7%
Blood volume*	—496 ml	—8%
Extracellular phase		
Extracellular water	—6.2 L	—36%
Total exchangeable sodium	—968 mEq	—32%
Total exchangeable chloride	—662 mEq	—30%
Intracellular phase		
Intracellular water	+1.0 L	+5%
Total exchangeable potassium	—114 mEq	—4%

*3 patients only

in total exchangeable sodium of 968 mEq or 32% of normal, and a decrease in total exchangeable chloride of 662 mEq or 30% of normal This reduction in extracellular water accounted for 75% of the total weight loss, and thus defined the main alteration in body composition as a loss of extracellular edema

The plasma volume showed a decrease of 295 ml or 9% of normal The red cell volume correspondingly was reduced with 197 ml or 7%

The intracellular phase did not lose any water On the contrary this phase gained 1 0 L of water or 5% of normal The total exchangeable potassium decreased by 114 mEq or 4% of normal

The decrease in total body water accounted to 5 2 L or 63% of the total weight loss The total weight loss could not be explained in terms of loss of preformed water alone, but it had to be assumed that the balance of the weight loss was made up by loss of body solids As the small decrease in total exchangeable potassium did not account for any larger loss of cellular tissue the balance of the weight loss must be assumed to be mainly due to an oxidation of fat during the recovery from edema

SUMMARY

The alterations in body composition during recovery from cardiac edema were measured as mainly a loss of extracellular water, sodium and chloride followed by a smaller reduction in plasma volume and red cell volume a small gain in intracellular water and a small decrease in total exchangeable potassium The total weight loss could only be explained with the assumption that oxidation of fat of a significant degree took place during the recovery from cardiac edema

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BLOOD AMMONIUM LEVELS AFTER INFUSIONS OF PROTEIN HYDROLYSATES*

MARY ANN PAYNE, NATHAN BROT, GEORGE JOHNSON, AND JOHN M BEAL

Metabolic balance studies in the immediate postoperative period have formed the basis for many conclusions regarding the detailed care of poor risk surgical patients Protein hydrolysates currently constitute the major source of nitrogen for these patients If the hydrolysates contain significant amounts of nitrogen which is not available for protein synthesis the nitrogen value used for the calculation of total protein should be corrected This paper will demonstrate the presence of significant amounts of ammonium nitrogen in the infused hydrolysate and will also show the effect of infusions of these hydrolysates on blood ammonium levels

Thirteen one liter infusions of 5% Aminosol in 5% dextrose were given over a 3 to 4 hour period to 12 patients on the surgical pavilion of The New York Hospital Ten of these patients were without evidence of liver disease or of other chronic disease, 2 had cirrhosis of the liver Venous blood samples were collected without stasis in an air free heparin ized syringe immediately before and after the completion of each infusion The pH was measured at 37°C on whole blood using a Cambridge pH meter Plasma ammonium nitrogen determinations were measured by a modification of the Seligson method,[1] within 20 minutes of venipuncture

The results of infusions of Aminosol into 2 patients without liver disease are shown in Table 1 There was a rise in venous blood ammonium nitrogen in all patients The greatest rise was 112 gamma % (patient 5), the average rise was 54 3 gamma % The ammonium nitrogen was measured in patient 7 two hours after the completion of the infusion and had returned to a base line value of 66 gamma % The pH values did not change significantly in any patient in this series

The results of three similar infusions of Aminosol in 2 patients with cirrhosis are shown in Table 2 The average rise in the blood ammonium nitrogen in these infusions was 147 6 gamma % The blood ammonium of the second patient rose 248 gamma % on the second infusion One hour after the completion of this infusion the ammonium nitrogen had already fallen to 186 gamma % and in 2 hours it had returned to a base line of 95 gamma %

Aminosol is an acid hydrolysate of beef blood fibrin Similar studies were undertaken using Amigen, a casein hydrolysate The results are shown in Table 3 The first 5 patients did not have any liver disease while the sixth patient had advanced cirrhosis with an elevation of the initial level of ammonium nitrogen The average rise of ammonium nitrogen for the first 4 patients was 81 2 gamma % The patient with cirrhosis showed a rise of 157 gamma % Again, the pH values did not show any consistent change

*From the Laboratories for Surgical Research, Departments of Surgery and Medicine, New York Hospital Cornell Medical Center, New York. Supported by research grants H 2261 and A181 from The National Heart Institute and National Institute of Arthritis and Metabolic Diseases of the National Institute of Health Public Health Service

Table 1 Plasma Ammonium – Nitrogen Values After One Liter Infusions of 5% Aminosol

PATIENTS WITHOUT LIVER DISEASE			
	AMMONIUM NITROGEN (GAMMA PER 100 ML)		
PATIENT	PRE INFUSION	POST INFUSION	Δ NH_4–N
1	50	157	+ 107
2	44	111	+ 67
3	85	132	+ 47
4	72	100	+ 28
5	79	191	+ 112
6	60	141	+ 81
7	69	94	+ 25
8	88	118	+ 30
9	64	78	+ 14
10	58	90	+ 32
Average			+ 54 3

Table 2 Plasma Ammonium – Nitrogen Values After One Liter Infusions of 5% Aminosol

PATIENTS WITH CIRRHOSIS			
	AMMONIUM NITROGEN (GAMMA PER 100 ML)		
PATIENT	PRE INFUSION	POST INFUSION	Δ NH_4–N
1	127	208	+ 81
2	93	207	+ 114
3	82	330	+ 248
Average			+ 147 6

Table 3 Plasma Ammonium – Nitrogen Values After One Liter Infusions of 5% Amigen

	AMMONIUM NITROGEN (GAMMA PER 100 ML)		
PATIENT	PRE INFUSION	POST INFUSION	Δ NH_4–N
1	96	150	+ 54
2	96	226	+ 130
3	88	150	+ 62
4	64	143	+ 79
5	180	337	+ 157

Two patients, one with a normal blood ammonium and one with an elevated initial level of ammonium, were infused with one liter each of 5% glucose in distilled water These control studies did not show any significant change in blood ammonium nitrogen

Freshly opened bottles of both protein hydrolysates were analyzed for nitrogen ammonium using the same micro diffusion technique The results were shown in Table 4 The average value of ammonium nitrogen for 5% Aminosol in 5% dextrose in distilled water was 53,100 ug % with a range from 42,000 to 70,000, for Amigen the average was 31,800 with a range from 26,000 to 36,000 gamma % ammonium nitrogen In comparison, 25% human serum albumin (Cutter) had only 332 gamma % and 1 day old bank blood 123 gamma % of ammonium nitrogen

It has been suggested that the ammonium nitrogen measured in protein hydrolysates might not be free ammonium but might be break down products of labile amides liberated *in vitro* by the strong alkali used in the micro-diffusion ammonium method In order to identify the source of this ammonium nitrogen, Aminosol was exposed to varying concentrations of sodium aluminum silicate (Permutit), an ion exchange resin The results of varying concentrations of Permutit on Aminosol are shown in Table 5 With sufficient time and exposure practically all of the reactive material is removed by the Permutit It follows, therefore, that the nitrogen measured in the hydrolysate is in the form of the ammonium ion

The presence of substantial amounts of ammonium in samples of protein hydrolysates and in the blood of patients infused with these hydrolysates has been pointed out recently by Webster and Davidson[2] Our results agree that the actual rise in blood ammonium after such infusions is high in the patient with cirrhosis and may easily enter the range of ammonium toxicity Our observations extend these studies and show that the values for ammonium nitrogen obtained on assay of hydrolysates are not a result of the breakdown of labile amides during the assay method but that ammonium nitrogen is present in the free state in the hydrolysate itself Therefore, the calculation of total protein based on the total nitrogen

Table 4 Ammonium Nitrogen Values of Protein Hydrolysates

	BOTTLES SAMPLED	AVERAGE AMMONIUM NITROGEN (GAMMA PER 100 ML.)
5% Aminosol	9	53100 Range 42000 70000
5% Amigen	4	31800 Range 26000 36000
0 9% Saline	3	18 Range 0 29

Table 5 Effect of Permutit on Plasma Ammonium Nitrogen Content of Aminosol

	PERMUTIT GM	AMMONIUM NITROGEN (GAMMA PER 100 ML.)
Aminosol	0	67000
Aminosol (100 cc.)	50	49

present in the sample may be misleading if correction is not made for ammonium nitrogen For example a liter of Aminosol contains about 7 3 gm of nitrogen Our assays show that 0 7 gm of this nitrogen is ammonium nitrogen Since ammonium nitrogen is not completely available to the metabolic pool[3 4] it should not be included in the calculation of total protein

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NUTRIODIALYSIS A NEW METHOD FOR ADMINISTRATION OF GLUCOSE AND AMINO ACIDS*

DEAN T GETTLER AND PAUL R SCHLOERB

Removal of accumulated toxic metabolites in uremia by the intestinal route has been investigated in this laboratory during the past 2 years[5] Perfusion of an isolated intestinal segment and intubation of the intestine with a closed cellophane tube have been evaluated in nephrectomized animals and uremic patients In the course of this study a closed cellophane tube containing an appropriate dialysate was inserted into the intestine and crystalloids were thereby dialyzed selectively from the blood via the intestinal lumen The ability of this technique to remove crystalloids has been confirmed

Vomiting associated with uremia may preclude administration of suffi cient dietary essentials to partially alleviate the uremic syndrome The administration of carbohydrate and amino acids by dialysis from the lumen of the cellophane tube into the intestinal lumen where they could be absorbed as an iso-osmolar and iso-electrolyte solution is termed nutrio dialysis

It is the purpose of this paper to present an experimental evaluation of this concept to compare the absorption by nutriodialysis with direct instillation and to suggest an explanation for the increased efficiency observed with nutriodialysis

METHOD

Female mongrel dogs were used for the operative procedures indicated A closed bag was made from cellophane tubing 20/32 inch in diameter

*From the Department of Surgery University of Kansas School of Medicine Supported by National Heart Institute U S Public Health Service Grant #H 2363

tied over the end of a polyethylene tube. Glucose determinations were done by a photometric adaptation of the Somogyi method by Nelson.[4] The amino acids were determined as nitrogen by the procedure of Koch and McMeekin.[3]

I. In vitro dialysis rates of glucose and amino acids. A cellophane bag 12 inches long, made from cellophane tubing 20/32 inch in diameter,* was used. The bag was filled with 48 ml. of an aqueous solution containing 46 gm. % of glucose and 6.5 gm. % of amino acids (casein hydrolysate**). The bag and contents were suspended in a one litre graduated cylinder filled with distilled water. Samples were taken from inside and outside the cellophane bag at intervals over a 4 hour period. Glucose and amino acids diffusing through the cellophane were determined.

II. The disappearance rates of glucose and amino acids from a closed cellophane tube inside the small intestine. The dogs used in this experiment and the experiments following were prepared by the standard method of making a Thiry-Vella loop of jejunum under sterile operative conditions. By this method an isolated segment of bowel was prepared with intact blood supply, and with proximal and distal ends opening through the abdominal wall. The dogs used had had the Thiry-Vella operation at least 4 weeks prior to beginning the experiment and were in good nutritional status, which was indicated by their maintenance of normal weight, continuing to eat the regular kennel diet, and absence of diarrhea and vomiting. The dogs were fasted overnight, weighed, and suspended in a harness. A cellophane bag measuring 20/32 inch in diameter and 18 inches long, was placed in the Thiry-Vella loop through the proximal stoma.

The dog (#67) used for glucose disappearance rate determinations had a three foot Thiry-Vella loop of jejunum. The bag was filled with 65 ml. of a 35 gm. % solution of glucose, containing 140 mEq. of Na, 4 mEq. of Ca, 2 mEq. of Mg, 105 mEq. of Cl, 4 mEq. of K and 42 mEq. of lactate per litre. Samples were removed from the cellophane bag at intervals, over a 4 hour period, and the amount of glucose diffusing through the cellophane was determined.

The dog (#24) used for the amino acid disappearance rate determinations had a seven foot Thiry-Vella loop of jejunum. The bag was filled with 70 ml. of an iso-electrolyte solution as described above, containing 8.9 gm. % amino acids (casein hydrolysate). Samples were removed at intervals, over a 4 hour period, and the amount of amino acid nitrogen diffusing through the cellophane was measured.

III. Comparison of direct instillation with nutriodialysis. A female mongrel dog (#127) was prepared with a three foot Thiry-Vella loop of jejunum. The dog, in good nutritional status, was fasted overnight, and anesthetized with sodium pentobarbital (30 mg./kg.). A cellophane bag 20/32 inch in diameter and 12 inches long was introduced into the proximal stoma. On 3 consecutive days 48 ml. of an iso-electrolyte solution, containing 26.8 gm. % glucose and 3.3 gm. % amino acids were introduced into the bag and allowed to remain for 4 hours. The solution in the cellophane bag and the drainage from the distal stoma were measured and sampled

*Obtained from the Visking Corporation, Chicago, Illinois.

**Stuart Amino Acids, obtained from the Stuart Company, Pasadena, Calif.

at the end of the 4 hour period Following this the intestinal segment was washed immediately with 200 ml of Ringer s solution The wash solution collected from the distal stoma, was measured and analyzed for amino acid nitrogen and glucose From these values the respective amounts absorbed were calculated

Using the same dog with the same preliminary preparation, a #24 Foley catheter was inserted into the proximal stoma and 46 ml of an iso elec trolyte solution, containing 23 gm % glucose and 2 8 gm % amino acids was allowed to drip into the intestine at a rate of approximately one ml /min for 50 minutes Since it was shown that when the nutrients are given by cellophane bag, most of them pass into the intestinal lumen during the first hour, the glucose and amino acids were given over a 1 hour period to make the rates of administration comparable The volume of solution collected from the distal stoma as drainage was measured and sampled at hourly intervals over a 4 hour period Following this, the intestinal segment was perfused immediately with 200 ml of Ringer s solution, and from the analyses for amino acid nitrogen and glucose, the absorption rates were similarly calculated

RESULTS

The data from the *in vitro* experiment relating to the appearance of glucose and amino acids outside the cellophane bag show that both glucose and amino acids pass readily through cellophane In this *in vitro* situation the rate of appearance of glucose outside the cellophane bag during the first hour was approximately 9 6 gm /linear foot of cellophane/hour and 3 3 gm the second hour The amino acid appearance rate outside the cellophane bag during the first hour was approximately 1 5 gm /linear foot of cellophane/hour and 0 15 gm the second hour

In the glucose dialysis study in the dog, during the first hour 14 5 gm of glucose diffused into the lumen Since the dog weighed approximately 12 kg, this would be furnishing glucose for absorption at 1 2 gm /kg /hour The subsequent disappearance rate was approximately 2 gm /hour

When the amino acids were placed in the cellophane bag in the intestinal loop, the amino acids diffused through the cellophane at a fairly constant rate, averaging 1 4 gm /hour over the first 3 hours

The data from the experiment comparing the absorption and drainage when glucose and amino acids were administered by direct instillation and then by nutriodialysis are shown in Table 1 When direct instillation was used there was drainage of 107 ml with absorption of 51% of the

Table 1 Comparison of Drainage and Absorption

	GLUCOSE ADMINISTERED (GM /4 HR)	AMINO ACIDS ADMINISTERED (GM /4 HR)	DRAINAGE (ML /4 HR)	GLUCOSE ABSORBED (%)	AMINO ACIDS ABSORBED (%)
Direct Instillation	10 6	1 3	107	51	46
Nutrio dialysis	11 1	1 4	15	93	79

glucose and 46% of the amino acids administered When nutriodialysis was used there was drainage of only 15 ml with absorption of 93% of the glucose and 79% of the amino acids administered

DISCUSSION

When nutrients such as glucose and amino acids are administered as hypertonic solutions directly into the gastro intestinal tract, the increased intra luminal osmolarity results in secretion of fluid, which may produce diarrhea with a loss of fluid and electrolytes and poor absorption of the nutrients A method of feeding which releases the nutrients into the lumen of the small intestine at approximately the rates at which they are absorbed might be expected to obviate this difficulty

The use of a cellophane bag is suggested as a method of regulating the release of glucose and amino acids The nutrients would have to pass through this semipermeable membrane before coming into contact with the intestinal mucosa

With these experimental conditions, the glucose transfer rate of 1 2 gm /kg /hour during the first hour by nutriodialysis approximates the total small intestinal absorption rate of 0 92 gm /kg /hour found in dogs by Trimble and Maddock[6] With the same experimental procedure, the transfer rate of amino acids of 1 4 gm /hour is in accord with the work of others According to McGee and Emery,[7] amino acids (hydrolyzed casein) in a concentration of 4 to 5% is fairly completely absorbed in 15 to 25 minutes when introduced into the small intestine of man by intestinal intubation Necheles and co workers[3] reported that when 1 gm /kg of body weight of amino acids was introduced into dogs by gastric intubation, the maximum absorption was in the first 30 minutes as indicated by the blood nitrogen reaching a maximum at that time, with return to normal in 3 hours This suggests that the amino acids were released from the bag at a rate which is near the absorption rate of the intestine

Preliminary results from these continuing studies suggest that when the same amounts of glucose and amino acids are administered by direct instillation and by nutriodialysis, better absorption and less fluid loss occurs with nutriodialysis

Application of this method to the nutritional maintenance of uremic patients with an isolated segment of intestine for perfusion is being investigated

SUMMARY

Nutriodialysis is presented as a method by which glucose and amino acids can be released into the lumen of the small intestine, by *diffusion* through a cellophane membrane, at rates near the maximum absorption rates of the intestine It was found by this method, that absorption of glucose and amino acids from short intestinal segments in dogs was feasible with minimal fluid transudation and net water loss

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A STABLE FAT EMULSION FOR CLINICAL USE*

EDWARD H STORER AND JOE CAMPBELL

Several fat emulsions for intravenous administration to humans have been developed in recent years In general these emulsions are prepared by high pressure homogenization of a suitable oil emulsifying agent and water Such aqueous emulsions have been shown to be well tolerated when fresh but reactions increase markedly with aging of these emulsions Most emulsions are unsatisfactory for human use afer one month and so far no aqueous emulsion has been acceptable beyond 4 months

Hydrolysis of the components very likely plays a major role in this deterioration of biological acceptability Accordingly about 3 years ago D B Zilversmit (Dept of Physiology Univ of Tenn) devised a method of preparing an anhydrous concentrate which is converted to an aqueous fat emulsion at the time of its use by the addition of water Soy bean phospatide is suspended in anhydrous glycerin by agitation in a Waring blendor This mixture is then placed in a colloid mill with a suitable oil and agitated until a satisfactory particle size is attained The resulting anhydrous concentrate or pre mix is then bottled autoclaved and stored At the time of administration an aqueous fat emulsion of the desired fat content is made by diluting the base with the appropriate amount of water or 5% dextrose solution

Physical stability of the anhydrous concentrate can be demonstrated by the following observations (1) a satisfactory particle size is still present after 2½ years of storage at room temperature (2) it is stable to auto claving and (3) it is stable to deep freezing (provided an unsaturated oil is used) Chemical stability is best shown by observing the rate of free fatty acid accumulation in the stored product This anhydrous concentrate hydrolyzes and thus liberates free fatty acids at about one tenth the rate of an aqueous emulsion Biological stability has been shown by intra venous administration of the stored product to humans One hundred and fifty six infusions of material from 4 to 14 months old have shown a reaction rate comparable to that for the freshly made emulsion

*From the Dept of Surgery University of Tennessee College of Medicine Memphis Supported in part by a grant from Abbott Laboratories North Chicago Ill

A year of animal experimentation preceded clinical testing. Our clinical experiences may be conveniently divided into three periods corresponding to the three different emulsion formulas used. The first formula in final dilution consisted of 10% fat, 0.5% phosphatide and 10% glycerin yielding about 760 calories per 500 cc. infusion. Three hundred ninety one infusions were given. The first 60 infusions were 1000 cc. each, the remainder, 500 cc. each. The reaction rate was rather high initially but by changing from sesame oil to cocoanut oil, and by slowing the rate of administration from 500 cc. in 1 hour to 500 cc. in 2½ hours an acceptable reaction rate was attained. The criteria for reactions are those suggested by the Surgeon General's Task Force on Intravenous Fat Emulsion.[1] Major reactions occurred in 4.6% of the infusions, minor reactions in 7%. Most of the major reactions consisted of intravascular hemolysis of sufficient magnitude to cause demonstrable hemoglobinuria. Such hemolysis makes this material unacceptable for human use.

A study to be reported elsewhere established glycerin as the cause of the hemolysis. It was also found that if the concentration of glycerin was lowered from 10% to 5%, significant hemolysis did not occur.

The second formula utilized the lowered glycerin content to avoid hemolysis and in final dilution consisted of cottonseed oil 10%, phosphatide 0.5% and glycerin 5%, yielding about 640 calories per 500 cc. infusion. The results are shown in Table 1. Although hemolysis was no longer a problem, the reaction rate was higher than would be acceptable in routine clinical use.

The third formula utilized the same anhydrous base as the second formula but the base was diluted with twice the volume of 5% dextrose in water solution. The final concentration was 5% cottonseed oil, 0.25%

Table 1. Reaction Rates with Different Dilutions of I.V. Fat

	10% FAT 189 INFUSIONS	5% FAT 65 INFUSIONS
Chill	8.0%	3%
Fever	7.5%	0%
Other	1.0%	0%
Totals	16.5%	3%

Table 2. Time Relations of 33 Reactions to 254 I.V. Fat Infusions

	ONSET DURING INFUSION	ONSET AFTER COMPLETION OF INFUSION	TOTALS
Chill	8	9	17
Fever	1	12	13
Other	0	2	2
Total	9 (3.5%)	23 (9%)	32 (12.5%)

phosphatide and 2 5% glycerin, yielding about 375 calories per 500 cc infusion Two chills and no other reactions occurred in 65 infusions (See Table 1) Both chills occurred in the same patient who received three infusions of this 5% emulsion This patient had no reaction to three infusions of the emulsion diluted still further to contain 2 5% fat

It is recognized that a 500 cc infusion yielding 375 calories has limited clinical value Accordingly postoperative patients are now being infused with 2000 cc of this 5% emulsion daily in lieu of the usual 5% dextrose in water The remainder of their fluid and electrolyte needs are supplied in a separate infusion Such a regimen provides a minimum of 1500 calories daily, more if a protein hydrolysate can be used as the third infusion

Table 2 demonstrates that patients receiving fat infusions must be observed for several hours following the infusion since the majority of reactions occur after the termination of the infusion

SUMMARY

1 A fat emulsion for clinical use is described This may be stored at room temperature as an anhydrous concentrate and is physically, chemically and biologically stable for several months

2 The results of 652 infusions of the anhydrous emulsion are discussed

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A SUMMARY OF CLINICAL EXPERIENCE WITH INTRAVENOUS FAT EMULSIONS*

HARRISON H SHOULDERS JR AND H C MENG

This report summarizes our clinical experience in the intravenous admin istration of a 30% neutral fat emulsion prepared in our laboratories[1] a 15% neutral fat emulsion Lipomul I V (10867) and an improved 15% neutral fat emulsion[2] Lipomul I V (11612) to patients on the general surgical service of Vanderbilt University and Thayer Veterans Administra

*From the Depts of Surgery and Physiology Vanderbilt University Medical School and Thayer Veterans Administration Hospital Nashville Tennessee Supported by the Research and Development Division Office of the Surgeon General Department of the Army Contract No DA-49 007 MD 252

[1] This emulsion contained cotton oil 30% glucose 5% soybean phosphatides 0.5% Tween 60 1 0% Demal 14 0.5% Ethofat C 15 1 0% Span 20 0.25% Aldo 28 1 0% and sodium cholate 0 1% and was prepared with pyrogen free water

[2] These emulsions were supplied by the Upjohn Co Kalamazoo Michigan and con tained cottonseed oil 15% glucose 4% soybean phosphatides 1.2% and pluronic F-68 0.3% Series 11612 differed from series 10867 only in that a cat blood pressure lowering factor was extracted from the phosphatides used

tion Hospitals The subjects comprised a wide selection of both pre operative and postoperative patients No patients were purposely excluded save those with recent coronary occlusion or other disorders from which sudden demise might reasonably be predicted

A 30% Emulsion This emulsion was administered up to 6 months after preparation and was stored at room temperature before use Its physical characteristics were excellent during storage the particle diameter averaging 1 micron or less One hundred fifty nine units of 300 ml each were given to 119 patients The 27 patients who received multiple infusions were given no more than 1 unit per day for a total of 5 units or less The duration of the infusion ranged from 2 5 to 4 hours (about 25 gm neutral fat per hour) Of the 159 infusions 119 were initial injections

The incidence of reactions was as follows thermogenic responses† of 2°F or more occurred in 27% of patients given initial injections and in 15% of those receiving multiple injections Shaking chills were observed in 3 instances Anaphylactoid responses lasting 5 to 10 minutes occurred in 6 cases characterized by dyspnea sense of chest oppression lumbar pain and facial flushing These reactions appeared as the first few drops were injected Thereafter, the initial rate was carefully regulated by diluting the first 5 or 10 ml of emulsion in a plastic delivery set with 5% glucose solution prior to injection After 20 minutes the rate was adjusted to 30 to 40 drops per minute In three instances in which the emulsion was begun very slowly with glucose flushing and slight dyspnea occurred which subsided within 5 minutes In these instances the emulsion was continued without further symptoms Two of the 6 patients described were asthmatics but no other disease could be correlated with the response A mild degree of nausea was noted in 5 patients a sensation of faintness in 2 pruritis in 2 patients and urticaria in 2 Careful measurements of plasma hemoglobin concentration showed no significant change

The thermogenic response characteristically began 1 to 2 hours after completion of an infusion reaching its maximum temperature 2 to 3 hours later The incidence of fever was not changed by slowing the rate of infusion to about 15 gm neutral fat per hour No difference in the incidence of thermogenic response was found in pre and postoperative groups

Twenty five patients each receiving a first infusion of this emulsion were alternately given [illegible] mg or a placebo There appeared to be [illegible] idence of febrile reactions in the two gro[illegible] bservations completed in patients receiving intravenous injections of hydrocortisone (100 mg) suggested that this agent lowered the incidence but did *not* prevent the febrile response

Serial studies of plasma electrolytes during intravenous infusions of the emulsion have been completed in 3 patients No change was observed in plasma sodium chloride and calcium concentrations Plasma potassium concentration diminished 10 to 15% of the control levels These findings

†Elevations in oral temperature were recorded as degrees above the highest temperature reached on the day preceding and following the day of infusion Readings were taken every hour for 7 hours after beginning the infusion

were confirmed in two experiments in dogs Since a decline in serum potassium concentration is known to occur during glucose infusion alone no conclusion can be drawn from this finding with respect to the effects of neutral fat

B Lipomul, I V (10867) Fifty two units of 600 ml each were given to 29 patients at a rate of about 18 to 20 gm per hour Of the 29 initial infusions in this group none was followed by an elevation above 2°F although 4 patients had elevations of 1 to 2°F Six patients received a total of 23 repeat infusions Of the 23 infusions 5 were followed by fever of 2 to 2 5°F all in patients with febrile courses The overall incidence of thermo genic responses above 2°F was 10% One patient an asthmatic experienced a severe anaphylactoid response identical with those noted above and one patient had a chill One patient became nauseated during the infusion No other untoward responses occurred

Blood pressure readings were taken at hourly intervals during 19 of the initial infusions Changes in blood pressure were variable The readings remained unchanged in 10 patients 6 were found to have systolic depression of about 20 mm Hg and 3 patients were found to have an elevation of about 20 mm Hg from the pre infusion level

C Lipomul (11612) Ninety five infusions of improved Lipomul were administered in 600 ml units to 55 patients at a rate of 20 gm neutral fat per hour Of this group one severe anaphylactoid reaction occurred in a young patient recovering from peritonitis but without evidence of hepatic disease or asthma The emulsion used in this instance had been kept for 5 months at room temperature The remaining 94 consecutive infusions were given using emulsions not over 2 months old which were kept refrigerated at 5 to 8°C prior to use No further reactions resembling the anaphylactoid response occurred The overall incidence of thermogenic responses above 2°F was 7% none reaching higher than 3°F while only 2% of patients receiving multiple infusions had this degree of fever No chills nor other reactions occurred in this group *Occasional instances of* subcutaneous infiltration have occurred with this emulsion as with the others The area involved became reddened and painful in 12 to 24 hours and resolved in another 2 days without treatment

The effects of intravenous heparin were studied in a group of 27 patients (age 30 to 65 yrs) using the improved Lipomul (11612) Each patient received the emulsion over a 4 hour period and a second infusion 2 to 3 days later Intravenous heparin was given in divided doses 100 mg at the beginning and 50 mg at the conclusion of the second infusion No untoward reaction occurred in these patients except for the febrile responses Three patients receiving the initial infusion had temperature elevations of 2 to 2 4°F When heparinized none of the 27 had temperature elevations above 2°F Four of the group were found to have 1 to 2°F elevation whether heparinized or not In this group of 27 patients heparin admin istration was not associated with any increase in degree or incidence of thermogenic response to the emulsion

Serial measurements of plasma unesterified fatty acids and total lipids were made in eight patients (age 30 to 40 yrs) of the heparin study group Plasma from these patients was shown to be capable of clearing neutral

Table 1 Effects of Heparin on Plasma Unesterified Fatty Acids and Total Lipids

	UNESTERIFIED FATTY ACIDS mEq/L ± S D			TOTAL LIPIDS mg % ± S D		
	NO HEPARIN	HEPARIN	P VALUE	NO HEPARIN	HEPARIN	P VALUE
Control	0.5391±0.2085	0.5688±0.3162	0.9	667±153	731±200	0.6
End of 4 hr Infusion	1.3228±0.4195	4.4638±1.3114	<0.001	1888±359	1596±233	<0.1
3 hrs after completion of infusion	0.6024±0.1304	1.1953±0.5167	<0.02	1422±301	798±197	<0.01

fat suspensions *in vitro* 3 minutes after the injection of 5 mg heparin. Five blood samples were drawn at intervals before during and after each infusion of emulsion. The results are shown in Table 1. When heparin was given the unesterified fatty acid concentration was significantly increased, and the level of plasma total lipids greatly diminished in comparison with values obtained when the patients received no heparin. Three hours after completion of the infusion plasma samples of the heparin treated group were visibly clearer than those from patients not given heparin.

THE INTRAVENOUS ADMINISTRATION OF GLYCEROL TO HUMANS*

HENRY A SLOVITER, CHARLES R SMART AND N HENRY MOSS

Human erythrocytes have been successfully preserved for more than a year[1] in a medium containing glycerol at below freezing temperatures. Erythrocytes which have been stored in this manner and have subsequently been freed of glycerol have been found to possess good viability as shown by post transfusion survival studies in human subjects.[2] Recently a method has been developed in this laboratory which permits the transfusion of thawed erythrocyte glycerol mixtures without completely removing the glycerol.[3] The use of this method for human transfusions has required the demonstration that significant quantities of glycerol can be safely administered intravenously to man.

It has been shown in experimental animals that administered glycerol is converted by the liver to glycogen and other carbohydrates.[4] It has been amply demonstrated that large quantities of glycerol can be administered

*From the Harrison Department of Surgical Research Schools of Medicine University of Pennsylvania Philadelphia. Supported in part by Grant H 1220 from the National Heart Institute National Institutes of Health U S Public Health Service

orally to experimental animals and to man without producing any undesirable effects[5, 6] However there have been reports that the parenteral administration of glycerol to experimental animals has been followed by hemoglobinuria[7] hypotension[5] and central nervous system disturbances[8] Critical examination of the results of these previous studies indicated to us that the toxic effects observed after the parenteral administration of glycerol might have been due to osmotic disturbances or dehydration caused by the injection of highly concentrated solutions of glycerol A recent study in this laboratory has shown that considerable quantities (2 to 3 gm /kg) of glycerol in relatively dilute solution can be administered intravenously to rabbits and dogs with the production of none of the untoward effects previously reported[9]

In the only previously reported instance of the parenteral administration of glycerol to man no noxious effects were observed after the intra arterial and intravenous injection of very small quantities[10] The present investigation deals with the intravenous administration of glycerol solutions to man and demonstrates that a considerable quantity of glycerol in a relatively dilute solution containing electrolyte can be infused without producing any undesirable effects

METHOD

The solution used for infusion was prepared for each patient by adding 50 gm of Merck Analytical Reagent Grade Glycerol to 1000 ml of sterile 5% glucose in 0 9% sodium chloride solution and then autoclaving the solution for 20 minutes at 15 lbs steam pressure The osmolarity of this solution is approximately four times that of plasma Each patient in the present series received 1 such unit of this solution containing 50 gm of glycerol by an intravenous infusion the duration of which varied from 3 to 6 hours Body temperature pulse rate respiratory rate and blood pressure were measured before during and after the infusion Urine samples collected before during and after each infusion were tested for the presence of hemoglobin by the benzidine test The patients were carefully observed and were asked if they experienced any unusual sensations

Each of a series of 12 patients received intravenously 50 gm of glycerol in solution as described above The patients all had malignant disease and were considered to have quite limited life expectancies They were in relatively stable condition and showed no gross abnormalities of their cardio respiratory urinary or central nervous systems

RESULTS

In most cases blood pressure was little changed from the pre infusion level during the course of the infusion The distribution of the maximal changes in systolic blood pressure from the pre infusion levels is shown in Figure 1 The diastolic pressure changes were smaller than the systolic changes It is evident that a small increase in blood pressure was more frequent than decrease and that the changes are no greater than might be expected from the relatively rapid intravenous administration of considerable amounts of fluid to older patients The maximal changes in cardiac rate from the pre infusion levels are also shown in Figure 1 and it is seen

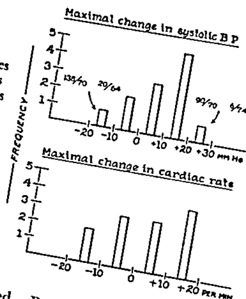


Fig 1 Distribution of the maximal changes from pre-infusion levels in systolic blood pressures and cardiac rates during the intravenous administration of a 5% glycerol 5% glucose 0 9% sodium chloride solution Above each case which showed the largest positive and negative change in blood pressure are shown the blood pressure values before infusion followed by the values of maximal change

that no significantly large changes occurred Body temperature showed no appreciable change which was ascribable to the glycerol for in no case was the temperature elevated more than one degree Fahrenheit There was no significant change in respiratory rate in any case

All the infusions were uneventfull with respect to any serious subjective reactions One patient remarked about a transient generalized feeling of warmth, another of nasal stuffiness of short duration and another that her legs felt cold for a few minutes There was no occurrence of headache, visual symptoms or dizziness No cardiac irregularities were observed In no case was it necessary to interrupt or discontinue the infusion before its completion There was no complaint of pain at the site of injection and no thrombosis of veins occurred

A moderate diuresis resulted from the infusion in most cases In all cases all urine specimens were found to be free of hemoglobin In one patient with pulmonary metastases whose chest was full of coarse râles there was significant clearing of the rales during the infusion This observation is noted as being of possible significance only because it has been reported that glycerol has a bronchodilating action [11]

SUMMARY

Human subjects have been given intravenously 50 gm of glycerol in a liter of solution which contained in addition to the glycerol 50 gm of glucose and 9 0 gm of sodium chloride No disturbances of cardiorespiratory or central nervous system function occurred, hemoglobinuria did not occur and no undesirable subjective effects were produced

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ALTERATIONS IN LEAN TISSUE AND BODY FAT ASSOCIATED WITH ANABOLIC HORMONE ADMINISTRATION*

HELENA GILDER, GEORGE N CORNELL GEORGE JOHNSON, JR, WILLIAM L CRAVER AND JOHN M BEAL

The potent anabolic effect of certain testosterone derivatives such as Norethandrolone (Nilevar®),† has been demonstrated previously in patients subjected to operation [1] These studies have been extended in 8 patients in an attempt to clarify the mechanism of protein conservation by this compound

METHOD

The experimental subjects consisted of 1 normal adult man (No 1) 2 female patients in the treated phase of thyrotoxicosis who were receiving the antithyroid drug Tapazole® (Nos 2 and 3) and 5 male patients who were subjected to partial gastrectomy for peptic ulcer (Nos 4 to 8) Sodium potassium and nitrogen balance data were collected on each patient Accurate body weights were recorded daily Oral intake was supplied in the normal subject and in 4 of the 5 operated patients as a formula the constituents of which were analyzed by batch to minimize intake error The remaining patients received accurately measured diets, the food content values for which were taken from standard dietetic tables Nitrogen and caloric intake were maintained at a constant level in the period immediately following surgery by intravenous protein hydrolysate for nitrogen and hypertonic glucose for calories Two of the patients also received a fat emulsion (Lipomul (I V) Upjohn & Co) intravenously The principles
Hospital Cornell Medical Center New York City Aided by grants in aid from the
have been reported in detail [2]

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*From the Laboratories for Surgical Research Department of Surgery The New York Hospital Cornell Medical Center New York City Aided by grants in aid from the Astor Foundation and from the Lillia Babbit Hyde Foundation

†Supplied by G D Searle & Co Chicago Ill

The anabolic hormone, Nilevar® (17 a ethyl 17 hydroxy 19 nor-4 androsten 3 one), was administered to 7 of the 8 subjects in doses of 25 to 50 mg It was given to subject No 1 intramuscularly and to patients Nos 2 and 3 orally for a period of 5 to 10 days after a control period without hormone The drug was administered intramuscularly to the operated patients 2 or more days prior to operation and was continued thereafter for the period indicated in the figures

CALCULATIONS

Daily lean tissue change was calculated by multiplying the nitrogen balance by 30

Daily changes in body fat were determined indirectly using a modification of Newburgh's formula [3] As originally derived by this worker, the formula for body fat change was based on an assumption that the calories expended by a patient to vaporize the water lost insensibly bore a constant relationship to the total number of calories burned Newburgh determined that the calories expended for insensible water loss represented 25% of the calories used by the patient More recent calorimeter studies by DuBois and associates[4] have demonstrated that approximately 31% of total calories burned are utilized in vaporization of water for subjects in an environmental temperature in the comfort zone (28° to 32°C) When this latter figure is applied to Newburgh's original expression for insensible water loss and the equation is solved for total fat burned (TF), the following formula is obtained

$$TF = IL - \frac{(2.6\ C + 15.1\ N)}{4.9}$$

Here IL is insensible weight loss and is derived from the patient's total intake, output, and the change in body weight, C is the total carbohydrate intake in grams, N is the total nitrogen excretion in grams The fat intake is assumed to be burned and is subtracted from the total fat (TF) to give a figure for body fat burned (BF) A negative value for fat (BF) indicates the storage of fat

RESULTS

Daily body weight change lean tissue change, and body fat change were calculated in terms of a body weight of 70 kg and were graphed cumulatively in Figure 1 for the 3 unoperated subjects and in Figure 2 for the 5 operated patients The average intake of nitrogen, total calories, and calories in the form of carbohydrate and fat for each patient are also recorded in the figures

Figure 1 demonstrates that the anabolic hormone consistently increased the rate of lean tissue storage but seemed to have a catabolic effect on body fat In subject No 1 and patient No 3 an actual loss of body fat occurred In patient No 2 the effect was reflected in a decrease in the rate of fat storage over that before the hormone was begun The weight gain of subject No 1 and patient No 2 exceeded the lean tissue gain during the period that the hormone was administered and suggests a retention of water

Of the 5 operated patients shown in Figure 2 the first, No 4, received no hormone This patient demonstrates the weight, lean tissue, and fat changes which usually result from the moderate trauma of partial gas

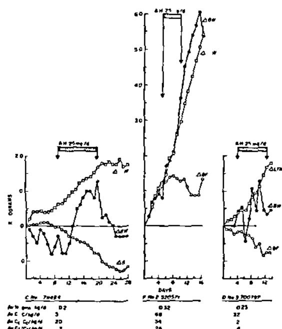


Fig 1 Cumulative body weight, lean tissue and fat changes in unoperated subjects C—No 1—711481, control, 26 yrs, initial body weight, (iBW)—71 kg, F—No 2—520571, thyrotoxicosis, 72 yrs, iBW—52 kg D—No 3—700797, thyrotoxicosis 57 yrs iBW—66 kg
A H = anabolic hormone, Nilevar® ΔBW = change in body weight ΔLTM = change in lean tissue mass ΔBF = change in body fat
Av N, Av C, Av C_c Av C_f = average intake in grams nitrogen total calories and calories in form carbohydrate or fat respectively

trectomy when an increased intake of nitrogen and calories are supplied It may be noted that there was a decrease of both lean tissue and body fat and that the sum of these two approximated the change in total body weight

Patients Nos 5 and 6 received 25 mg of anabolic hormone, a dose which gave minimal but definite protection from the typical lean tissue loss after operation As with the unoperated subjects, there was in patient No 6 immediately postoperatively a divergence between the lean tissue and the fat changes, i e a large loss of body fat was accompanied by minimal decrease in the lean tissue mass

The effect of anabolic hormone on lean tissue and fat, noted above in operated patients, was most apparent in patients Nos 7 and 8 who received the larger dose of the hormone Here, as with the unoperated patients there was an increase in lean tissue mass and a substantial decrease in body fat Patient No 7 showed an extraordinary storage of lean tissue in the postoperative period, a result which frequently occurs in patients such as

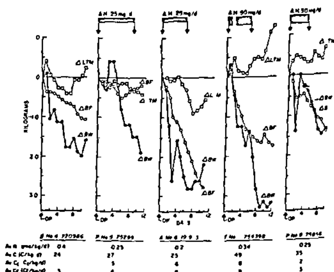


Fig 2 Cumulative body weight lean tissue and fat changes in operated patients
B—No 4—720986 42 yrs, iBW—70 kg
P—No 5—752997, 60 yrs, iBW—57 kg
A—No 6—191973 50 yrs, iBW—67 kg
T—No 7—754398 58 yrs iBW—46 kg
Pa—No 8—758567, 57 yrs, iBW—56 kg
For other symbols see Figure 1

this one who are depleted prior to operation Patient No 8 however, was not depleted and the lean tissue gain was the result of the high nitrogen and caloric intake postoperatively combined with the administration of the anabolic hormone

COMMENTS

The accuracy of the method here described for determining changes in body fat has not as yet been fully defined and will require the study of a greater number of subjects before it can be stated with certainty

Changes in body water have not been mentioned here The measurement of total exchangeable sodium (TES) to estimate changes in extracellular water has been utilized and where there was a discrepancy between the sum of the lean tissue and body fat changes, and the change of total body weight the difference was accounted for by a change in body water indicated by TES Such was the case in patient No 7 (Fig 2) whose weight loss in the 6th to 10th postoperative days was found to be due to a water loss

The data presented here suggests that the anabolic hormone enhances lean tissue storage at the expense of fat Balance studies should be done to determine whether fat is required or, as is most probable is merely the only available source of energy The fact that in patient No 2 the rate of storage of body fat was reduced but not eliminated during hormone administration indicates that the oral intake of fat and carbohydrate was sufficient to supply energy for the anabolic action Patient No 3 on the other hand received insufficient oral calories for the process and burned body fat There may be some danger therefore in administering the anabolic hormone to severely malnourished patients unless it is possible to supply sufficient calories so that the patients will not need to call on his own meagre fat stores

The administration of the hormone to surgical patients who have adequate fat stores with a sufficient intake of both nitrogen and calories may reduce the loss of body protein characteristic of the postoperative period and thereby decrease the morbidity in patients recovering from operations of maximum trauma

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Wound Healing and Infection

THE HEALING OF HUMAN WOUNDS*

IN VIVO STUDIES

HAROLD B HALEY AND MARTIN B WILLIAMSON

At present there is no reliable method for quantitatively measuring the rate of healing in humans The concept of *rate* implies several measurements made at different times Thus, the use of tensile strength or biopsy methods for a determination of rate of healing would have obvious and very serious limitations If a quantitative technique for serially measuring a property related to the healing of wounds were available it might be used to expand our knowledge in such directions as (a) the fundamental processes which are involved, (b) the various factors which influence these processes (nutritional, hormonal, etc), and (c) the abnormalities (dehiscence, stasis ulcers, keloids, etc) which are observed clinically This paper is a report of some of the steps which have been taken to develop such a technique, based on the use of the isotope labeled amino acids which have been shown to be intimately involved in the processes of wound healing

The considerable investigations of the healing processes in experimental animals undertaken in our laboratory provide the basis for the current study It has been shown that cystine is the limiting amino acid required for wound healing [1, 2] Since methionine can be converted to cystine *in vivo*, both are expected to have and do exhibit the same effect in accelerating the rate of healing [3, 4] When the cystine, methionine and nitrogen content of healing wound tissue is plotted against the time of healing the rate of deposition of cystine is very much greater than that of the methionine or nitrogen and appears to be proportional to the rate of healing as measured by tensile strength determinations Analogous experiments using sulfur 35 labeled amino acids have given similar results [4, 5]

The main element of strength in the healing wound resides in the collagen fibers which contain essentially no methionine or cystine [6] The cystine content of the regenerating wound tissue must then presumably be found in the fibroblasts It would follow, therefore that studies of the sulfur amino acid metabolism are concerned with the active mechanism of wound healing rather than with the relatively inert end product of healing collagen

METHOD

It was expected that the deposition of S^{35} in the surface of the healing wound could be measured after the administration of S^{35} labeled cystine or methionine to surgical patients A number of problems were encountered

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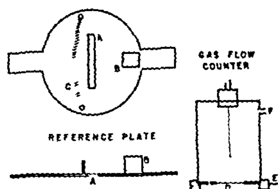


Fig 1 Apparatus for obtaining radioactivity measurements in the superficial layers of regenerating wound tissue in humans *A* open slot in "reference plate *B* positioning block to accept positioning pin in gas flow counter *C* spring to hold gas flow counter in position *D* metallized Mylar window *E* positioning pin *F* inlet for counting gas

and had to be solved to reach this objective That sulfur 35 is a so-called soft beta emitter (0 167 m e v) has been an advantage from the standpoint of safety to the patient However the low energy of the S^{35} emission has made the quantitative determination of the radioisotope more difficult In order to measure the radiation a gas flow counter was devised as shown in Figure 1 One of the principal difficulties met was to obtain reproducible measurements from day to day This entails obtaining a reproducible geometry which has two aspects measuring the radioactivity in the same place on the wound on successive days and having an identical vertical distance from the incision to the window of the gas flow counter each time These difficulties were overcome by the use of a brass reference plate [7] This reference plate screens out all the radiation coming from the subject except that through the rectangular slot The slot is positioned over the part of the incision where the measurement is to be made It is held in position by adhesive tape placed over the two lateral extension arms Skin pencil marks around the plate indicate the area for setting the plate on subsequent days With the reference plate set down firmly the incision bellies slightly through the slot

When the reference plate was properly in place over the incision the gas flow counter was attached to it as explained in Figure 1 The width of the slot of the reference plate was designed to be somewhat greater than the width of the healing incision necessitating that a correction be made to compensate for the nonregenerating tissue included in the counting field This was accomplished by the use of the following equation

$$W_c = C_w - \frac{A - A_w}{A} C_s$$

where W_c is the radiation from the regenerating wound tissue in counts per minute C_w is the counts per minute measured in the area of the incision exposed by the reference plate slot C_s is the counts per minute measured when the slot exposed only skin tissue A_w is the area of the regenerating wound tissue exposed by the slot and A is the total area exposed by the slot

RESULTS

Using the technique described we have measured the S^{35} activity in the superficial layers of the incisions of 12 patients after feeding 1 0 *millicuries* of either methionine-S^{35} or cystine S^{35} 24 hours preoperatively All of the patients underwent partial gastrectomy for ulcer The results obtained

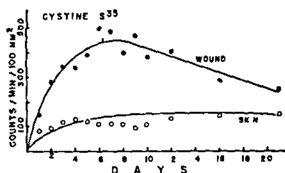


Fig 2 The sulfur 35 activity in the superficial layer of healing incisions and skin when gastrectomy patients are fed 1 0 millicuries of cystine S^{35} 24 hours before operation All values are corrected for decay

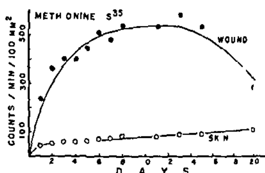


Fig 3 The sulfur 35 activity in the superficial layer of healing incisions and skin when gastrectomy patients are fed 1 0 millicuries of methionine S^{35} 24 hours pre operatively All values are corrected for decay

are indicated in Figures 2 and 3 The radioactivity of the skin was measured on the thigh The sharp drop in sulfur 35 activity found almost directly adjacent to the incision made the activity in this area comparable to that observed at more distant sites such as the thigh or the forearm

SUMMARY

An outline of the experimental background and current development of the work on a quantitative method for determining the rate of healing in humans is presented The method depends on the serial measurement of the radioactivity which appears in the healing wounds of surgical patients after the administration of S^{3} labeled cystine or methionine

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THE VASCULAR BASIS FOR TENDON REPAIR*

Erle E. Peacock, Jr.

Many individual characteristics of a tissue, particularly features of healing, can be explained by peculiarities of blood supply. In spite of difficulties frequently encountered in repair of tendon lacerations, there has been almost no investigation of circulation in these structures. In view of continuing enthusiasm to find some substance to wrap around tendon grafts, it seems important to determine the significance of individual vessels in the ultimate fate of a graft.

Kolliker,[1] in 1850 stated that tendons have practically no blood supply. Mayer,[4] in 1916, described blood vessels around tendons, particularly the mesotendon, but the only basic investigation and complete description of blood vessels entering tendons was published by Edwards[2] in 1946. He injected the vessels with sodium nitroprusside benzidine and demonstrated a rich longitudinally oriented vascular pattern. Unfortunately, after having demonstrated these vessels, he assigned them no importance and finished his paper with a return to the old concept that tendons are static structures with virtually no metabolic function or vascular requirements. Braithwaite[3] injected colloidal silver iodide solution into an amputated digit that contained an old flexor tendon graft and demonstrated a profuse circulation in the postoperative adhesions.

Edwards and Braithwaite demonstrated three main groups of blood vessels which are apparently the same for both normal tendon and autogenous grafts. These are the vessels that enter at the musculotendinous origin, the tendinoperiosteal insertion, and the intermediate vessels which enter through the mesotendon and vincula. The anatomical demonstration of these vessels challenges the concept that tendons can be treated as a nonviable substance and the antithesis of this concept can be proven by a demonstration of the effect of occlusion of specific vessels.

METHOD

The question of whether tendon has an active metabolism can be settled by determining the metabolic activity of tendon slices in the Warburg apparatus. Such values are not available in standard tables of tissue metabolism and accordingly we made slices of human tendon from various locations and subjected them to this analysis. The average QO_2 for tendon is about 1 microliters of oxygen/mg. dry weight/hour. This is small compared to highly active tissues such as liver or retina, but definite enough to suggest that the blood vessels in tendons are purposeful structures necessary for a measurable metabolism.

Our next step was to determine the importance of each group of vessels and the competency of anastomosis between them. Radioactive phosphorous uptake was selected as the method to investigate this and proved to be a sensitive indicator of minute circulation. The drug was injected into four different animal preparations using a dose of 6 microcuries/kg. After the

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phosphorous had circulated freely for an hour segments of tendon were taken at regular intervals along its length and were counted in a utility scaler with a manual sample changer Background counts were in the range of 34 to 45 counts per minute and the presence of phosphorous produced a count of from 85 to 150 per minute In this investigation we were interested only in qualitative results Either the phosphorous was present indicating an active circulation or it was absent indicating no effective circulation in that segment of the tendon In all of the experiments a free tendon was placed in the wound during the period of phosphorous circulation to serve as a control for surface contamination The experiments were performed on adult dogs and both flexor and extensor tendons of different sizes in both extremities were studied The results were constant for all tendons over 8 cm in length which most nearly corresponds to flexor tendons in human beings Four different preparations were studied to determine the effectiveness of each of the three vessel groups and of the vessels formed in postoperative adhesions

To test circulation at the musculotendinous origin a tendon was detached from its periosteal insertion and from all of the intermediate vessels along its course The tendon was gently suspended above the wound to prevent radioactive contamination by blood and serum and was kept warm and moist with physiological saline The only intact blood vessels were those entering and leaving through its musculotendinous origin Radioactive phosphorous was injected into the general circulation and one hour later the tendon was removed and transverse sections made at regular intervals along its length Radioactivity was definite in the proximal one third but in none of the tendons was there any radioactivity in the distal two thirds Apparently there was no effective anastomosis between vessels entering at the musculotendinous end of the tendon and vessels in the distal two thirds

In a second experiment the same type of preparation was used except the proximal end of the tendon and the intermediate vessels were divided leaving the tendon suspended only from its periosteal insertion Again radioactivity was present in the tendon but in this situation only the distal one fourth was radioactive Both of these experiments give functional significance to Edwards anatomical demonstration that the vessels entering at either end of a tendon are no larger or more numerous than the centrally located intrinsic vessels Thus one can say that vessels entering at the end of a tendon are not adequate to nourish the entire tendon and that the intermediate vessels must be of vital importance

A third experiment was designed to show the function of the intermediate vessels This experiment was similar to the first two except that both ends of the tendon were cut and the intermediate vessels retained In this situation since the tendon could not be suspended out of the wound a free piece of tendon was placed beside it to serve as a control for surface contamination The tendon with intact mesotendon was three times more radioactive than the control and the radioactivity was evenly distributed throughout Actually the counts in these preparations were nearly the same as the counts in undisturbed tendons and indicate that the inter

mediate vessels can provide a normal circulation in the absence of vessels entering from either end

A final experiment in this series consisted of stripping away the intermediate vessels and replacing the tendon in its bed for 5 days At the time of radioactive phosphorous injection the proximal and distal ends of the tendon were severed from their junction with muscle and bone The tendon was attached to the body only by 5 day old surgical adhesions Again, the tendon showed marked radioactivity evenly distributed throughout This experiment indicates that 5 day old *postoperative adhesions* effectively connect the intrinsic blood vessels of a free graft with those of the host

From the radioactivity experiments it appears conclusive that blood vessels entering either end of a tendon cannot alone nourish its center Furthermore, postoperative adhesions carry vessels capable of nourishing an entire tendon graft or transfer This suggests a serious objection to the practice of wrapping a tendon graft in an artificial sheath to prevent *adhesions Skin, blood vessels,* plastics, amnion, and allantoic membranes have all been proposed for this purpose

To demonstrate further the importance of intermediate vessels and what can be expected when they are prevented from reforming, polyethylene sheaths were placed around flexor and extensor tendons To accomplish this polyethylene tubes were split down one side and placed around tendons without disturbing their muscular or periosteal attachments The slits in the tubes were sealed with liquid plastic before the overlying skin edges were sutured Twelve days later the wounds were

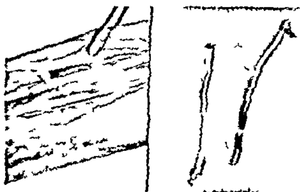


Fig 1 Polyethylene tubes surrounding flexor tendons in a dog's forearm On the right the same tendons are seen 10 days later The left tendon shows typical central necrosis and the right tendon shows distal necrosis as well presumably due to a separation of the distal suture line

re-opened and the proximal and distal thirds of the tendon were found to be perfectly normal within the sheath The center third was necrotic however, again demonstrating the need for intermediate centrally located blood vessels When an intact plastic tube was slipped over one end of the tendon and the periosteal end resutured to bone or if both ends were divided as in a free graft the same result was obtained In this type of preparation blood vessels could grow into *either end of the tendon and* nourish the proximal and distal thirds but a mechanical barrier to adhesions in the center prevented the re-establishment of circulation to this area *The same result could be obtained by sliding a short* metal ring up and down the tendon daily In this experiment the ring was advanced up and down the tendon by steel wires brought out through the skin above

and below the sutured wound The ring severed all of the central adhesions each time it was moved, and by the end of 2 weeks the center of the graft was necrotic

DISCUSSION

To recapitulate, long tendons have an active and measurable metabolic rate and to maintain this activity are provided with a definite and constant vascular supply The intrinsic vessels are longitudinally oriented and are supplied by extrinsic vessels at both ends and through the mesotendon in the center The vessels at either end are no larger or more numerous than elsewhere and do not nourish more than one third of the length of the tendon We postulate that tendon transfers and free grafts heal somewhat like free split thickness skin grafts Because of their low metabolic rate, under favorable circumstances they can survive 3 or 4 days by diffusion of gases and nutrients from the host The largest aggregation of cells in a tendon is to be found in the visceral layer of the sheath and this, of course is in direct contact with the capillaries of the host For complete success particularly if gliding function is to be preserved, vessels of the graft must actually connect with vessels of the host and this includes the intermediate segmental vessels as well as those entering from either end If one is successful in shielding the graft by a mechanical barrier so that post operative adhesions cannot form, necrosis of the center of the graft will occur Grafts which survive and retain the properties of a normal gliding tendon are nourished by fibrous adhesions The elasticity and attenuation of adhesions is variable, just as the character and amount of surface scarring varies in individuals and this may be one explanation why some grafts eventually glide after a period of stretching On the other hand there is the all too familiar experience of performing a technically perfect operation in a cooperative patient only to find the graft solidly incarcerated in dense, unyielding cicatrix, and these patients may be comparable to the superficial hypertrophic scar formers following surface restorations In view of the peculiarities of the circulation to tendon grafts, it would seem the investigation of the production and alteration of collagen is more likely to be rewarding than mechanical attempts to prevent adhesions

SUMMARY

Tendons are not structures which can be indiscriminately moved with no regard for their metabolic requirements or blood supply Tendons have a low but measurable metabolic rate and depend upon certain definite blood vessels for nourishment Vessels entering from either end are not adequate to supply the center of a tendon and the intermediate segmental vessels found normally in the mesotendon and postoperatively in adhesions are of vital importance Mechanical prevention of adhesions will deprive a graft of this important fraction of its blood supply and ultimately result in necrosis of the center of the graft

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CHEMICAL AND METABOLIC STUDIES IN WOUND HEALING IN MAN*

PHILLIP E LEAR BYRON M TREITLER AND CHARLOTTE MANDELL

This study is motivated by our deep concern about wound disruption in the postoperative patient and the serious clinical problems which follow this complication We have always felt that wound disruption was related more to the healing power of the sutured structures than to either the type of suture (continuous interrupted figure of 8 etc) or the type of suture material (silk cotton catgut wire etc) We are studying healing at the fascial level in man Our patients are unselected cases undergoing abdominal surgery We hope to establish some concept of the norm and feel that if our series gets large enough we will have within it some cases of wound disruption Data on such cases we hope may shed some light on the basic problem

High power magnification of fascia reveals fibroblasts lying in a homogeneous matrix This homogeneous matrix under electron microscopy reveals collagen fibrils lying in the ground substance With x ray diffraction studies the collagen fibrils can be seen to be composed of proto fibrils Bear[1] considers these proto fibrils to be single peptide chains He describes them as a long thin single crystal and as such its formation or dispersion synthesis or degradation is expected to be largely at the mercy of its surroundings When methods for examination of the chemical and physical structure of these crystals are designed these may furnish information regarding the physiological milieu from which they are derived

The ground substance is a complex mucopolysaccharide The ultimate source of this material has not been defined but is considered to be secreted by the fibroblast or mast cells The fibroblasts also secrete a protein substance which may be what some workers call procollagen or tropo collagen This procollagen in the mucopolysaccharide matrix is with the aid of a complex enzyme system converted into collagen fibrils These fibrils are the ultimate holding structures when a wound is sutured Ascorbic acid is used in wound healing either by its effect on ground substance or the enzyme systems It is necessary for the hydroxylation of

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*From the Department of Surgery State University of New York College of Medicine at New York City The Ray and Charles Newman Surgical Research Laboratory Long Island Jewish Hospital Supported by a research grant (RG-4393 R) from the National Institutes of Health Public Health Service

Extremely valuable help was given in this work by Dr Paul Gallop Biophysicist and Dr Morton Schwartz Biochemist

proline into hydroxyproline which is one of the four main constituents of collagen fibrils Ascorbic acid is not intimately related to the adult collagen fibril In scorbutic animals, with poor wound healing, the collagen fibrils appear normal but there is a marked change in the ground substance

Our studies included examination of the blood for various protein fractions, fascia removed at operation for collagen content, urine for various nitrogenous products, and, the area of the healing wound by means of Ivalon sponge technique We discontinued blood studies because they were nonrevealing The results were similar to results in previous work in our laboratory on tissue protein studies We found that the circulating plasma can rob the tissue stores of protein to maintain "false" values for circulating plasma proteins Fascia studies for collagen were limited because many patients could not spare fascia in amounts necessary for the examination The area of healing wound and the urine were studied as follows 12 unselected patients from the surgical service were studied They were patients with gallbladder disease, herniae, peptic ulcer and elective large bowel surgery These patients were placed on a hydroxyproline free diet for 3 days before operation On the day of operation they were nourished intravenously, and where indicated, postoperatively As soon as the patient tolerated a return to the hydroxyproline free diet, this was ordered This diet excluded meat, fish, gelatin, ice cream and candy This allowed the use of the whole group of dairy proteins, carbohydrates and fats

Hydroxyproline is one of the four commonest aminoacids in collagen and one of the easiest compounds to identify in a qualitative and quantitative way Progress in collagen formation (and likewise wound healing) might be studied by studying the metabolism of hydroxyproline Proline

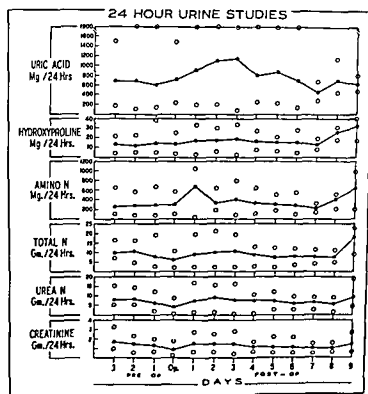


Fig 1. 24 hour urine studies Heavy line is one typical patient Small circles represent high and low for the series

and hydroxyproline, in some assays, constitute 25% of the aminoacids in collagen

Twenty four hour urine specimens were collected in the standard manner for the entire period of the study (usually 14 days) These specimens were examined for urea, uric acid, creatinine, alpha amino nitrogen, hydroxyproline and total nitrogen Figure 1 represents the values in a typical patient and the high and low values for the series Total nitrogen excretion is similar to other data reported in the literature, with a peak rise on the first to second postoperative day Urea nitrogen follows this curve closely and thus total nitrogen may be a reflection of urea excretion These changes are probably related to tissue catabolism and stress mobilization Aminoacid nitrogen and hydroxyproline rise gradually after surgery, reaching a peak on the third postoperative day, and then gradually return to normal At the peak, these values are about three times the preoperative levels If this increased excretion reflects mobilization or synthesis of hydroxyproline for purposes of collagen formation, it is not reflected at these early stages in the healing wound tissues (see Ivalon sponge data below) Uric acid excretion increases and reaches a high about the third postoperative day There is a concomitant rise in serum uric acid in these patients Such increases have been reported after administration of ACTH and hemorrhage Response to the stress situation of surgery is a likely cause of this increase in these patients Creatinine studies are not particularly revealing

To obtain data on tissue changes at the wound site, small pieces of Ivalon sponge (an inert polyethylene compound) about ½ x 1 x 2 cm were sutured into the end of a 1 cm penrose drain with a 3 0 silk suture Four such Ivalon sponges were placed in contact with the suture line of the fascia and were placed about 3 cm apart The drains were thus in the milieu of physiological activity of the healing wound These drains were removed on various postoperative days They were removed without difficulty and produced no ill effect on the patient or on the healing wound All wounds to date have healed per primum and are solid

Immediately on removal, the Ivalon sponges were sent to the laboratory in sealed glass containers They were examined immediately or frozen until such time as they could be examined Prior to chemically extracting the sponge, a thin slice was removed for preparation of histologic sections These sections indicated that the process of repair at the operative site is reflected in the interstices of the sponge Figures 2a, b, c, d, e represent sections of plain sponge, and sponges removed at the 3rd, 5th, 7th and 10th day from a patient One notes increasing fibroblastic activity and fibrin formation

The remainder of the sponge was divided into two portions One portion was used to study the collagen content This was done by assay for hydroxyproline The other portion was used to study the ground substance by measuring hexosamine content Hydroxyproline determinations were done according to Neuman and Logan [7] Hexosamine determinations were done according to Rondle and Morgan [8] Blank runs on the sponge revealed no detectable evidence of hydroxyproline or hexosamine Nitrogen values were



Fig 2a Photomicrograph of section of Ivalon sponge (X230)



Fig 2b Photomicrograph of section of Ivalon sponge removed 3 days postoperatively (X540) Note occasional fibroblast lining septum of the sponge Remainder of sponge infiltrated with red and white blood cells



Fig 2c Photomicrograph of section of Ivalon sponge (X540) removed 5 days postoperatively Notice increased number of fibroblasts lining sponge septum



Fig 2d Photomicrograph of section of Ivalon sponge (X540) removed 7 days postoperatively Now fibroblasts are seen and there are a few fibres and a few giant cells visible

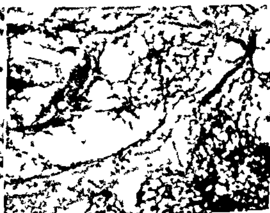


Fig 2e Photomicrograph of section of Ivalon sponge (X540) removed on 10th postoperative day Note extensive deposit of fibres in interstices of sponge

determined in the extracts and results reported as milligram of hydroxyproline or hexosamine per milligram of nitrogen

Figure 3 is a summary of the hydroxyproline and hexosamine data in this study The heavy line represents a typical patient and the small circles extremes for the series There is no marked rise in the hydroxyproline content of the sponges after (10 day) contact with the healing wound There was one exception in the series The only patient in the group who was explored for a second look and had recurrent carcinoma had an unusually high value for hydroxyproline on the 5th day postoperatively and this returned to the 3 day level by the 7th postoperative day The alkaline extract from the 7 day sponge in this patient jelled

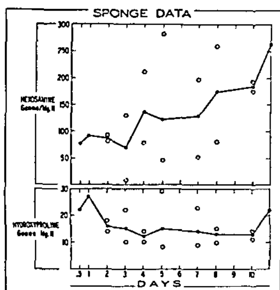


Fig 3 Hydroxyproline and hexosamine analyses of sponge extracts Curve is the average for this group Small circles represent high and low values for the series

when it reached room temperature, but was easily liquified on application of heat or addition of small amounts of trypsin We have no explanation for this phenomenon

There is an increase in the hexosamine values throughout the period of contact of the sponge with the healing wound

These figures differ from the results reported in rats by Dunphy and Udupa[4] They reported a marked hydroxyproline rise after the 8th day and reaching a level of almost 300% increase by the 16th day Their values for hexosamine rose to a peak on the third day and rapidly dropped to operative levels by the 10th day We were reluctant to leave sponges in the human wound too long but if we had we might have found our data to correspond to that obtained in animals for hydroxyproline The hexosamine differences we would at present have to explain on metabolic differences between animal and man

It is interesting to note that practically all of the published studies on the basic nature of ground substance and collagen have been done on animal tissues Human fascia and tendon are easily obtainable and one wonders why the workers in this field have avoided using human tissues

We hope to continue this work and feel that when the series is large enough statistically we will ultimately have some patients with wound disruption in the series The data on such patients should prove interesting

SUMMARY

Wound healing is related to the physiological milieu of the healing area and not the type of suture or suture material

A method of studying this milieu in man has been described Further studies will be carried out in an effort to find the type of milieu which may predispose to dehiscence of the postoperative wound

Studies will then be made to determine how an improper milieu may be anticipated and corrected preoperatively to prevent dehiscence

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HORMONAL INFLUENCE ON HEALING WOUNDS THE EFFECT OF ADRENALECTOMY AND CORTISONE ON THE QUANTITY AND COLLAGEN CONTENT OF GRANULATION TISSUE*

LOUIS N. PERNOKAS, LEON C EDWARDS, AND J ENGLEBERT DUNPHY

Clinical and experimental evidence is gradually being accumulated to suggest that regenerating as well as stable connective tissue may be affected by certain systemic hormones The effect of the adrenal hormones in particular has been the subject of much investigation The present study was undertaken to delineate certain chemical changes produced in regenerating connective tissue by adrenal steroids

Evidence regarding the effect of adrenalectomy on granulation tissue and healing wounds is conflicting Chassin and Localio[1] found that in adrenalectomized rats there was an increase in the tensile strength of 5 day old laparotomy wounds Taubenhaus[2] has described a suppresion of granulation tissue around turpentine abscesses in adrenalectomized animals On the other hand, there is general agreement that cortisone has an inhibiting action on granulation tissue, although opinions differ as to its site of action [3] It is possible that methodology enters into the conflicting reports The sponge biopsy technique[4] was used to obtain the data to be presented here

METHOD

The experiments were performed on young male albino rats weighing between 150 to 200 gm All animals were maintained on Purina rat pellets and water *ad libitum* The adrenalectomized animals were given 1% saline solution instead of water No attempt was made to pair feed the animals They were watched closely, however, and although those receiving cortisone did not eat as much as the controls, they continued to be active, bright eyed, and in no way appeared ill

Polyvinyl alcohol pledgets (Ivalon) were implanted subcutaneously in 1 cm incisions under light ether anesthesia along the back of the animals The wounds were closed with wire sutures Infection was not a problem but any sample which grossly or histologically appeared septic was dis

*From the Department of Surgery, Harvard Medical School, The Fifth Surgical Service and Sears Surgical Laboratory Boston City Hospital Supported in part by a research grant (RG 3949 C) from the National Institutes of Health Public Health Service and Damon Runyon Memorial Fund for Cancer Research

carded Pledgets were removed on the 4th, 8th, 12th, and 20th day after implantation The animals were divided into six groups (1) control (2) adrenalectomized and maintained on saline 1% (3) adrenalectomized and maintained on 2 5 mg cortisone daily (4) adrenalectomized and maintained on 5 mg cortisone daily (5) intact animals given 2 5 mg cortisone daily (6) intact animals given 5 mg cortisone daily Cortisone was administered to all groups intraperitoneally The hormone produced no peritonitis and apparently was absorbed without difficulty

The tissue samples removed in the sponge were vacuum dried and the total dry tissue produced was determined by weight in comparison with the pre implantation weight of the sponge The tissue was then analysed for hydroxyproline by a modified method of Neuman and Logan from which the total collagen content was computed

RESULTS

The results are shown in Figures 1, 2, and 3 † Figure 1 is a graphic representation of the total amount of dry granulation tissue produced per 100 mg of sponge It shows that, for the most part in all the groups the tissue produced was similar to the control animals Although more work is required to determine the significance of a few variations, it is interesting to note that at 8 days the adrenalectomized animals appear to have produced more granulations than the controls

Figure 2 presents the collagen produced in grams percent of new dry tissue It reflects the relationship of total collagen to total dry tissue produced in 100 mg of sponge, and demonstrates that the adrenalectomized group may be slightly less efficient in collagen production than the controls The cortisone treated groups are definitely less efficient and as the dosage is increased from 2 5 mg to 5 mg daily the efficiency decreases correspondingly

Figure 3 depicts collagen in grams percent of new dry tissue produced in the adrenalectomized and cortisone treated animals The group receiving 2 5 mg of cortisone daily continues to have a low collagen content at 8 days but by 12 days the curve has reached that of the controls Surprisingly the group receiving 5 mg daily has a curve superimposed on that of the control animals

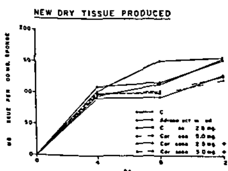


Fig 1

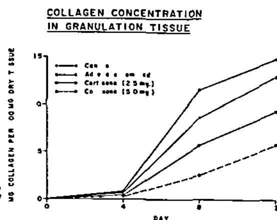


Fig 2

†Each point on the graphs represents the mean of ten or more determinations on 5 different animals

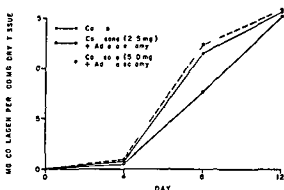


Fig 3

SUMMARY

From a preliminary study of the effect of adrenalectomy and cortisone on granulation tissue produced in polyvinyl alcohol sponges the following conclusions may be inferred Adrenalectomy does not significantly affect production of collagen or granulation tissue Cortisone will decrease collagen production significantly at dosages which do not appear to affect total granulation tissue production This effect of cortisone even in doses of 5 mg, which is far above the physiological level was not demonstrable in the adrenalectomized animal

CONCLUSION

This study is interpreted as supporting the concept that regenerating connective tissue is under direct or indirect control of a balance of systemic hormones and that the effect of cortisone is not a specific pharmacologic phenomenom Further studies are in progress

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ALTERATIONS IN SERUM GLUTAMIC OXALACETIC TRANSAMINASE ACTIVITY FOLLOWING OPERATIONS*

William L. Craver, George Johnson, Jr., and John M. Beal

Serum glutamic oxalacetic transaminase activity increases in patients with myocardial infarction, liver necrosis, or skeletal muscle injury. A transitory elevation in the serum transaminase level has been found after operations. The present study was undertaken to define the magnitude and duration of this postoperative rise.

Glutamic-oxalacetic transaminase, or transaminase, is a specific tissue enzyme. It is concerned with the transfer of the alpha amino nitrogen of aspartic acid to alpha ketoglutaric acid which results in the synthesis of glutamic acid and oxalacetic acid. The normal serum level in man ranges between 8 and 40 units per milliliter per minute (U/ml/min). The highest tissue level is in the heart muscle. It is next highest in skeletal muscle. Brain, kidney, liver, testes, lung and spleen follow in that order.[1]

The serum transaminase remains normal in most disease states. When tissue injury occurs there is a decrease in transaminase activity in the injured tissue associated with an increased activity in the serum. These findings were first applied to the study of cardiac and liver diseases. Transaminase activity in the serum rises within 12 hours after a myocardial infarction, and thus is a useful test for detection of myocardial infarction in its early stages. Liver cell injury from toxins or infections caused marked elevation in the enzyme level. Ligation of arteries which cause infarction of various tissues in experimental animals has been reported to produce elevation in serum transaminase activity.[2] An increase in the serum transaminase level has been found after various surgical procedures in man and in experimental animals.[3,4]

METHOD

Studies were made on a group of 20 patients who were subjected to various surgical procedures. At least two preoperative venous blood specimens for serum transaminase determinations were drawn, which included one immediately before the induction of anesthesia. Blood samples were obtained at the termination of the operation and every 2 hours during the first 6 to 10 hours after operation. Thereafter, a blood sample was taken every 24 hours until transaminase activity returned to control levels. All patients in this study had normal preoperative levels. The transaminase activity in the specimen was determined with the spectrophotometric method of Karmen.[5] Since serial dilution of the serum in the reaction mixture will increase the transaminase titer, it is imperative that the same volume of serum be used for any given patient when the test is repeated at various intervals. One half milliliter of serum was used in all determinations.

Electrocardiograms were obtained before and after operation in most of the patients and evidence of myocardial infarction was absent. Liver

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function was tested by means of alkaline phosphatase, thymol turbidity bilirubin, and bromsulphalein excretion in the majority of the patients Significant alterations were not found

RESULTS

This investigation confirms that a rise in serum transaminase activity is the usual event following surgery The elevation is transitory The maximum values are reached within the first few postoperative hours and a rapid decline follows However, control levels often are not reached for several days If blood samples are not drawn until the morning after operation, the period of greatest rise in activity will not be demonstrated

The alterations in transaminase activity in 4 patients in this series are illustrated in Figure 1 The first patient (NYH #353742) had a multiple abdominal organ resection (partial pancreatectomy, partial gastrectomy, left adrenolectomy and splenectomy) for carcinoma of the pancreas Serum transaminase activity reached a peak of 207 U /ml /min 2 hours after operation The level fell sharply but did not return to normal until the fourth postoperative day

A rise to 137 U /ml /min occurred in the second patient (NYH #492795) within 3 hours after a total gastrectomy through a thoracoabdominal incision Transaminase activity returned to normal by the fifth post operative day

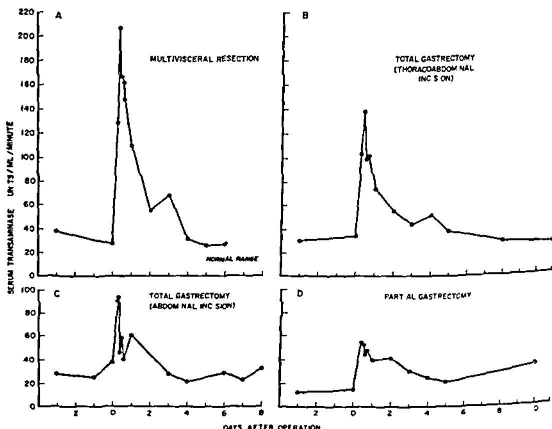


Fig 1

The third example is a patient (NYH #766148) who had a total gastrectomy in which a transabdominal approach was used. The postoperative rise was of lesser magnitude, a maximum of 92 U./ml./min. 2 hours after operation.

The fourth patient (NYH #740029) had a partial gastrectomy and with this less extensive operation, the serum transaminase rose to 55 U./ml./min 1 hour after surgery. The response of 3 other patients who were studied after partial gastrectomy resembled that illustrated in Figure 1 (Table 1).

The postoperative rise in transaminase activity in all the patients in this series (Table 1) was proportional to the magnitude of the operative procedure, with only one exception. The exception was a debilitated man who had a multivisceral resection for carcinoma of the colon. The serum transaminase level did not rise above 38 U./ml./min. in this individual.

The effect of general anesthesia with minimal trauma was assessed in 4 patients who had minor urological procedures under general anesthesia. Transaminase activity did not rise significantly in these patients.

DISCUSSION

A pattern of rapid, transient elevation of serum transaminase activity has been found after operation The magnitude and duration of the rise

Table 1. Alterations in Serum Transaminase After Operations

OPERATION	NUMBER OF PATIENTS	RISE IN TRANSAMINASE	
		MAXIMUM	HRS AFTER OP
Multivisceral resection	2	207	2
		38	2
Esophageal resection	1	158	4
Total gastrectomy			
a Thoracoabdominal incision	1	137	3
b Abdominal incision	1	92	2
Partial gastrectomy and choledocotomy	1	105	2
Right Hemicolectomy	1	82	2
Partial Gastrectomy	4	70	7
		63	2
		61	0
		55	1
Repair of hiatus hernia	1	67	0
Cholecystectomy	2	67	5
		63	2
Sigmoid resection	1	53	4
Sigmoid polypectomy	1	36	4
Cystoscopy	3	42	9
		26	3
		23	4
Urethral dilitation	1	29	4

appears to be roughly proportional to the extent of the operative procedure

The absence of evidence of myocardial damage and liver dysfunction in association with a sharp rise and fall in transaminase activity suggests that the postsurgical increase in serum transaminase activity is due to skeletal muscle and other tissue necrosis caused by surgical trauma

Since myocardial infarction does occur during or shortly after operative procedures it is useful to realize that operation alone may produce a significant elevation in serum transaminase activity

SUMMARY

A transitory elevation in the serum glutamic oxalacetic transaminase level occurs after operations The maximum values are found within the first few postoperative hours The magnitude of trauma influences the height and duration of the rise in serum transaminase activity

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EFFECT OF NITROGEN MUSTARD AND THIO TEPA ON WOUND HEALING*

J HAROLD CONN SAMUEL M LEB AND JAMES D HARDY

The long search for an effective carcinolytic chemotherapeutic drug may be closer to realization with the clinical use of some of the newer alkylating agents Two groups of drugs the phosphoramides and the nitrogen mustards are of particular significance They have demonstrated their effectiveness in the palliation of bronchogenic carcinoma [3] mycosis fungoides [1] neuroblastoma [9] Hodgkin s disease [10] lymphosarcoma [4, 10] and chronic leukemia [8, 10] At the present time chemotherapy is indicated when these neoplasms become refractory to roentgen therapy or when the disease is too widely disseminated for the effective irradiation of the involved organs

Further extension of the use of alkylating agents is envisioned as an adjuvant to the surgical resection of certain neoplasms Such use renders

*From the Departments of Surgery Veterans Administration Center and University of Mississippi Medical Center Jackson Dr Elizabeth Ferrington Assistant Chief of Pathology interpreted the histological sections used in this study

it desirable to learn the effect of these compounds upon wound healing, for the effect of alkylating agents on normal and neoplastic tissue is similar to that of ionizing radiation They both cause inhibition of mitosis, frag mentation of the cell nucleus and eventual destruction of the cell

The action of the mustards as described by Gilman and Philips[4,7] depends upon intramollecular cyclization with formation of a series of ethylenimonium compounds The latter are highly reactive and are capable of alkylating a large number of biological functional groups such as sulfhydryl, amino, sulphide, carboxyl, and organic phosphate It is probable that by acting with one or more such groups the mustards produce a deleterious effect upon intracellular proteins The cytotoxic action may be the result of inactivation of one or more cellular enzymes

The activity of triethylene thiophosphoramide (Thio-TEPA) is depend ent upon its contained ethylamine residuals Hendry *et al*,[5] from results obtained with ethylamine derivatives, attributed their cytotoxic action primarily to changes produced in the chromosomes and preferentially directed toward the cells undergoing division This action, however, is not limited to malignant cells, but extends to rapidly proliferating cells of all types

What then, is the effect of nitrogen mustard and Thio TEPA on the rapidly proliferating fibroblasts, angioblasts, and other cells involved in wound healing? Must the administration of these drugs be delayed until wound healing has progressed sufficiently so as to avoid the possibility of wound disruption? Or can they be given at operation? Theoretically, it would be advantageous to start the drugs at the time of operation with the objective of destroying the neoplastic cells squeezed out into the peri pheral circulation[6] Clearly, it becomes necessary to know at what stage in the operative period the chemotherapeutic drugs can safely be given so as to achieve their maximum therapeutic effect without interfering with normal wound healing The following experiments were devised to examine the question

METHOD

Thirty apparently healthy fasting mongrel dogs were anesthetized with intravenous pentothal and the abdomens opened through an upper midline incision under sterile conditions Standard 5 cm incisions were made in the gastric antrum anteriorly, paralleling the lesser curvature These incisions were closed with a single continuous inverting 4 0 silk suture The abdominal walls were closed with continuous 2 0 silk On the seventh postoperative day the animals were sacrificed and the stomachs removed The silk suture was removed from the gastrotomy wounds, and the wounds disrupted with air pressure which was measured with a mercury manometer The healing wounds were removed for histological study

Ten dogs were used as controls 10 were given nitrogen mustard intra venously, 0 4 mg /kg of body weight and 10 were given Thio TEPA intramuscularly 2 0 mg /kg The total dose of each drug was divided into three equal parts and given on successive days, starting on the day of operation Neither the abdominal nor the gastric wounds of any of the dogs became infected The seventh postoperative day was chosen for observation in this study because it occupies a strategic position in the

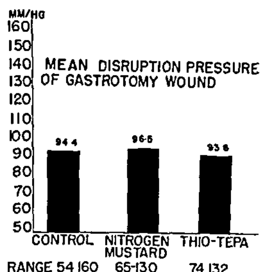


Fig 1

healing curve It is located on that portion of the curve which slopes sharply upward, and any delay in fibroplasia is detected at this point with greater consistency than either the very early or very late phase of healing

RESULTS

The results are summarized in Figure 1 The wound disruption pressure of the control group varied from a high of 160 mm of mercury to a low of 54 mm of mercury, with a mean of 94 4 mm The nitrogen mustard group showed a high of 130 mm of mercury, low of 65 mm, with the mean 96 5 mm The Thio TEPA group ranged from a high of 132 mm to a low of 74 mm, with the mean 93 6 mm Microscopic sections of wounds removed from the three groups revealed no essential difference in histological picture

DISCUSSION

The fact that these drugs produced no demonstrable effect on wound healing seems a little surprising, as the basis for their therapeutic use is the cytotoxic and growth inhibiting effect they have on rapidly proliferating cells This seeming paradox may be explained by Block and Murphy[1] in their work with nitrogen mustard They state that the major part of the destruction of susceptible cells (lymphocytes, microcytes, erythroblasts, and megakaryocytes) occurs in the hemopoetic tissues, not in peripheral blood Moreover, the fixed cells (fibroblasts, microphages, and reticular cells), as well as plasmacytes, are extremely resistant to the destructive action of the mustards This, of course, applies also to Thio TEPA and the other ehylamines, as their action is essentially similar to that of the nitrogen mustards This is further supported by the fact that histological sections from the wounds of the treated animals in this series showed normal fibroplasia and reticulum formation, with no evidence of inhibition of fibroblastic activity

CONCLUSIONS

1 Nitrogen mustard (methyl bis beta chloroethyl aminehydrochloride) and Thio-TEPA (triethylene thiophosphoramide) administered in recommended dosage caused no essential difference in wound healing either

grossly or histologically, from normal controls in dogs

2 From experimental evidence it appears feasible to start treatment with these chemotherapeutic drugs on the day of operation to obtain their maximum therapeutic effect without fear of wound healing failure

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EFFECTS OF THRIETHYLENEMELAMINE ON WOUND HEALING*

EDWARD T KREMENTZ W R GIDDENS W L CHAPMAN

The current use of the various alkylating agents as adjuvants to definitive surgery of cancer or for palliation following incomplete removal of malignant tissue is becoming widespread One concern is that these toxic drugs will cause an increase in the local and systemic complications associated with surgery

We became interested several years ago in the effect of the chemotherapeutic agents on wound healing following the abdominal dehiscence of a patient with a nonresectable adenocarcinoma of the stomach This 77 year old colored man at the Pineville Charity Hospital was given a 4 day course of HN (1 mg kg) starting on the fifth postoperative day The evisceration occurred on the ninth postoperative day and a discussion

*From the Department of Surgery The Tulane University School of Medicine Supported in part by the Cancer Teaching Grant CT 62 from the National Cancer Institute of the National Institutes of Health Public Health Service and by The Anna Fuller Fund

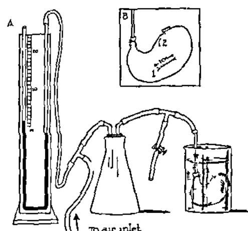


Fig 1 *A* Apparatus for testing bursting pressures of animal stomachs *B* (1) Site of wound (2) Usual point of rupture exclusive of the wound

ensued as to whether or not administration of HN_2 had a causal effect in this wound complication The following experiment was undertaken to help elucidate the presenting problem

METHOD

Healthy adult guinea pigs weighing from 500 to 800 gm were used for the study The animals were fed on Purina rabbit chow, fresh greens, and water *ad libitum* and were housed in wire cages in an air conditioned room The animals were fasted for 36 hours prior to surgery and on the first postoperative day, although they were allowed to have water during this period The animals were anesthetized with intraperitoneal pentobarbital sodium, Abbott (25 mg/kg) and supplementary ether inhalation Originally HN_2 was selected as the test agent, however as it is not practical to give this drug intravenously in the guinea pig, a supply of triethylenemelamine (Lederle) was obtained to give orally This did not prove feasible so the drug was given in solution intraperitoneally The test animals were given 4 consecutive intraperitoneal injections of triethylenemelamine (25 mg/kg) beginning on the second postoperative day No untoward reactions were observed from this route of administration

The method of testing as described by Harvey[4] is designed to measure the bursting strength of a gastrotomy wound in millimeters of mercury Sterile technique was used throughout The stomach was delivered through a small midline incision in the upper anterior abdominal wall A 1 cm longitudinal incision was made in the distal anterior surface of the stomach in a relatively avascular area The incision was immediately closed with a continuous running suture of #80 black silk inverting the mucosa A second layer of 3 to 5 interrupted Lembert sutures of #80 silk was then placed to approximate the serosa The stomach was replaced and the abdomen was closed in layers with interrupted sutures

In the second group a slightly different technique was used as the experiment was conducted by another operator and #60 cotton sutures were used instead of silk

On the seventh postoperative day the animals were sacrificed and the stomachs were carefully removed All adhesions about the gastric wound were removed with the stomach and the gastroesophageal junction was ligated As indicated in Figure 1, each stomach was attached to a system

Table 1 Effect of T E M on the Bursting Pressures of Gastrotomy Wounds

GROUP 1 GUINEA PIGS			GROUP 2 GUINEA PIGS	
CONTROL mm Hg	TEST mm Hg		CONTROL mm Hg	TEST mm Hg
36	30		36	13
32	22		54	10
38	22		40	20
30	22		42	44
22	23		30	42
40	24		34	22
30	25		42	30
26	34		40	20
34	26		30	28
42	34		44	30
30	20		38	46
32	25		38	—
38	-		44	
33 1	25 6	Average	39 4	27 7
Difference 7.5 mm Hg			Difference 11 4 mm Hg	
t Test 01> P> 001 mm Hg			t Test 01 > P > 001 mm Hg	

for recording air pressure The stomach was then inflated slowly until the wound site or the stomach ruptured and the bursting pressure was recorded as an index of wound strength

In Group 1, microscopic sections of several of the stomach wounds were studied In Group 2, sections of all abdominal and stomach wounds were carefully examined

RESULTS

In Group 1, 13 control and 12 test guinea pigs were used The average bursting pressure of the stomachs in the controls was 33 1 mm Hg and the average bursting pressure for the test animals was 25 6 mm Hg, a difference of 7 5 mm Hg The t test indicated the difference to be highly significant

In Group 2, 13 control and 11 test guinea pigs were used The surgical technique was somewhat different from Group 1 therefore, it was felt that the results should be analyzed statistically as a separate group The average bursting pressure of the stomachs in the controls was 39 4 mm Hg and for the test animals was 27 7 mm Hg, a difference of 11 7 mm Hg As in the first group the t test again indicates the difference to be highly significant

The following observations were recorded in the examination of the microscopic sections of the gastrotomy and abdominal wounds 1) epitheli zation of the wounds 2) presence of mitoses, 3) amount and appearance

of the fibroblastic response, 4) the presence of acute and chronic inflammatory cells, 5) the type and extent of foreign body reaction, i e to sutures Mitoses were noted to be present in all sections The extent of the wound reaction varied considerably but could not be correlated with the bursting pressures There was some tendency to note more reaction in the weaker wounds but this was not a constant finding

DISCUSSION

It is known that T E M , as shown by Plummer, *et al*,[6] in concentrations causing a therapeutic effect in humans, completely inhibits mitosis in chick fibroblast tissue cultures for 24 hours or longer Although the mitosis was stopped, the migratory ability of the cells was not affected Nitrogen mustard probably acts in an identical way[3, 4] and similar results were obtained in comparable experiments on the corneal epithelium[2] The ability of the drug to retard mitosis suggests that the rate or quality of wound healing might be diminished

From the results of our studies, the impression is that triethylenemelamine delayed the healing response in some manner and made the stomachs of the test animals more prone to rupture No obvious cause for inhibition of wound healing was apparent by the gross or microscopic inspection of the wounds

It must be noted that the method used of measuring wound strength is an indirect one and, as pointed out by Taylor,[7] the size of the stomach is an important factor This is true according to the principle that if pressure within a hollow container is constant the force on the wall varies as does the radius Thus, given two stomachs of equal strength but different size, the larger stomach will burst at a lower pressure than the smaller This principle must be recognized although with random selection of a large number of animals of a uniform size, the error would be minimal

The dosage of T E M in guinea pigs may be high as compared to that used in humans The usual individual course is from 15 to 40 mg This amounts to a total dosage of 0 2 to 0 6 mg /kg[1] The guinea pigs received 1 mg /kg per course and it is noted that this is the LD 50 for mice[5] In preliminary experiments in which 17 guinea pigs were treated, deaths occurred in only 2 animals, these being the result of operative complications The weights were recorded in a number of guinea pigs and while the individual weight loss varied from 0 to 200 gm , the average percent loss of body weight was less than 10% In 3 animals leukocyte counts were recorded with the above dosage The drug caused a fall from 15,000 to 5000 white counts in about 8 days, after which time they began to rise It was felt that the amount of drug given produced a vigorous but not excessive therapeutic response

SUMMARY AND CONCLUSIONS

1 The bursting pressures of gastrotomy wounds were determined on the seventh postoperative day in guinea pigs receiving daily intraperitoneal doses of triethylenemelamine (25 mg /kg) from the second to the fifth postoperative day Bursting pressures were also obtained on the operated control animals

2 The postoperative administration of T E M to guinea pigs caused a significant depression in wound healing as determined by bursting pressures

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STANDARDIZATION TEST FOR SUTURES*

J W BLUNT, JR.

The standards for absorbable surgical sutures are to be found in the Pharmacopeia of the United States,[1] in the 15th revision These are the standards used by the manufacturers in preparing the sutures and by the Food and Drug Administration to protect the surgical profession from inferior products The standards are specified for the length, diameter, and tensile strength of the absorbable material, they specify that each of these determinations shall be made of a suture "immediately after removal from the container, without stretching and without drying" The standards also define the amount of soluble chromium compounds that can be present, the sterility requirements the packaging and storage containers for the sutures, and what must be placed on the label of the suture These specifications are fine for the manufacturer, the controller, and the seller of these materials, but they do not aid the surgeon in determining the properties that he needs to know when selecting an absorbable suture material It is the purpose of this paper to suggest another standard, the half strength time of absorbable sutures in animal tissue

The Scott inclined plane tester was used for all tensile strengths Type C chromic, absorbable surgical suture, sizes 1 0, 2 0, 3 0, and 4 0, was purchased through jobbers from the various established suture manufacturers The types that were tested were the non boilable variety and were packaged in non glass containers A random sample was taken from each batch and tested for tensile strength under the specifications as given in

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the Pharmacopeia Another sample of packages was opened and the strand divided in the middle Both halves were handled by standard operating room techniques one was then coiled and placed in a container of sterile saline for 1 hour prior to determining its tensile strength The other half of the suture was placed in the subcutaneous tissue of white rats It was possible to implant four different brands of sutures in the same animal At intervals the animals were sacrificed the sutures recovered and their tensile strengths tested It was possible to plot percentage of strength against time and thereby derive a half strength time for each suture

Since the coarser grades of absorbable suture do not lend themselves to use in the rat the studies were confined to sizes 1 0 2 0 3 0 and 4 0 chromic C catgut After 1 hour immersion in saline the tensile strength of a suture is reduced to approximately 75% of its fresh strength After 5 days in the rat the tensile strength of the sutures had dropped to below 50% of the saline value The half strength time for all four sizes fell between 4½ days and 5 days When an arbitrary time of 4 *days was set for the half strength time approximately two thirds of the* brands tested had longer half lives than this and approximately one third fell below this half strength time When re calculated for individual sutures it was found that 75% of the strands had longer half strength times than 4 days

DISCUSSION

In surgical practice an absorbable suture is never used for its ultimate purpose immediately after removal from the container it is immersed in fluid at times before the surgeon gets it and always in the fluids of the tissues where he places it Therefore the arbitrary time of one hour of saline immersion was chosen as a starting point for the tensile strength tests *Since the curve of loss of tensile strength* has a variable shape it was felt that using the half time would be a convenient measuring point

At present we are working on *in vitro* concoctions using bovine plasma with various additive enzymes to attempt to derive a half strength time for *in vitro* testing which will correspond to the half strength time for the *in vivo* work In the past a trypsin digestion test has been proposed [2] but since we have never been able to identify trypsin in a healing wound other than in minute quantities it seems best to use one of the other enzyme systems

The reason for establishing this half strength time is twofold 1) to check the figures that are presented by the various suture manufacturers for their products before recommending them for use in our hospital 2) in the near future it is anticipated *that we will have synthetic absorbable sutures* which will eventually replace the conventional catgut and other direct collagen byproduct materials We needed to establish a surgical standard for the presently available materials against which we could compare these absorbable synthetic surgical sutures

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PROLONGED HYPOTHERMIA IN EXPERIMENTAL PNEUMOCOCCAL PERITONITIS*

R S WOTKYNS H HIROSE, AND B EISEMAN

Generalized body hypothermia has been utilized in numerous isolated instances in the management of various infections, but rarely has there been scientific evaluation of its therapeutic value[1,2,3,4] The rationale for the use of this modality in the management of infection depends upon the fact that growth of bacteria pathogenic to man is depressed at temperatures below 37°C The host benefits from hypothermia, however, only if bacterial growth is diminished less than host resistance

This is an analysis of the course of pneumococcal peritonitis in mice maintained at 21°C for 24 hours compared to simultaneously infected normothermic control animals The first portion of the study compares length of survival, utilizing an overwhelming inoculum lethal to all animals, the second compares absolute survival utilizing an inoculum calculated to produce an LD_{50} in the normothermic controls, and the third portion of the study is an analysis of the effect of hypothermia plus antibiotic therapy on the course of the experimental infection

METHOD

A total of 770, 6 to 8 week old (25 to 30 gm) white mice were used in this study Each animal received a 0 5 ml intraperitoneal injection of an 18 hour culture of Pneumococcus III diluted as noted below The virulence of the organism was fastidiously maintained by subcultural and animal passage techniques[5,6] Two hours after inoculation the animals to be cooled were narcotized with phenobarbital were rapidly cooled in a 4°C room for 6 to 8 minutes and were maintained in a constant temperature compartment at a steady body temperature of 21° to 24°C for 24 hours Rectal temperatures were recorded by a constantin thermacouple attached to a Brown potentiometer At the end of the 24 hour period the animals were removed from the cold box and allowed to warm spontaneously at room temperature Normally this took 1 to 2 hours The control mice were maintained at room temperature with adequate available food and water

Group 1 This group of 329 mice received 0 5 ml of undiluted 18 hour broth culture of Pneumococcus III — a dose designed to kill the normothermic control animals in 48 hours One hundred and sixty animals were cooled 130 served as paired simultaneous normothermic controls and 39 control animals were anesthetized but not cooled

Group 2 The inoculum in this group of 263 animals was sufficiently dilute (0 5 ml 10^{-5}) to produce approximately an LD_{50} in the normothermic controls and thus to differentiate absolute survival between the hypothermic and normothermic animals One hundred and three animals were cooled 142 animals were normothermic controls and 18 mice were anesthetized but not cooled

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Group 3. The 178 animals in this group were given an inoculation of 0 5 ml of a 3 x 10 ⁻ dilution of an 18 hour broth culture and then were divided into four groups Ninety animals were each given an intramuscular injection of 400 units of sodium penicillin 2 hours after their experimental infection, 47 animals were then narcotized and cooled while 43 animals served as normothermic but penicillin treated controls The other group of 88 animals received no penicillin, 45 animals being cooled in the usual manner, and 43 serving as simultaneous untreated normothermic controls

In each study an occasional animal died during the 24 hour period of hypothermia from causes (overdose of anesthesia, drowning etc) other than pneumococcal peritonitis as proven by autopsy, and are not included in the study

The *in vitro* rate of growth of the organism at 37°C and 22°C was compared by serially measuring the turbidity of broth cultures maintained at these temperatures

RESULTS

Group 1. Effect of Hypothermia on Survival Following Infection with a Massive Dose of Pneumococci. Figure 1 illustrates the results of this study and shows that the hypothermic group survived approximately 48 hours longer than the normothermic control group

Group 2 Effect of Hypothermia on Survival Following Infection with an LD_{50} The pattern of survival in this group is illustrated in Figure 2 and shows that hypothermia favorably alters mean survival from 38% in the controls to 61%, a difference of 23%

Group 3 Effect of Hypothermia plus Penicillin on Survival Figure 3 shows the effect of the combination of hypothermia and penicillin compared to the three groups of simultaneous controls It is evident that hypothermia does not significantly alter the infection in animals given antibiotic coverage Both modalities have a therapeutic effect but their actions are not synergistic

Effect of Cold in Vitro Growth of Pneumococcus III. *In vitro* growth of Pneumococcus in the cold was significantly retarded resulting in a decreased number of organisms and in prolonging the lag phase of the growth curve

Effect of Anesthesia on Survival of Normothermic Infected Animals Anesthesia as here employed did not significantly alter the infection

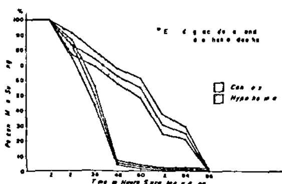


Fig 1 Animal Survival Following Massive Infection

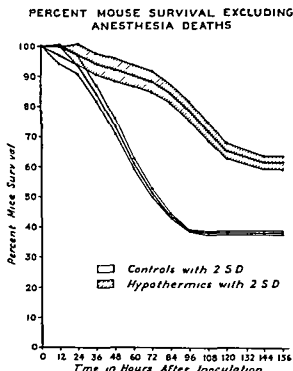


Fig 2 Animal Survival Following Sublethal Infection

DISCUSSION

These experimental studies indicate that the course of a severe infection can be significantly altered by hypothermia When an overwhelming infection is employed the time of survival is prolonged When a sublethal dose is used mean survival can be increased 28% Under the conditions employed, hypothermia had no appreciable additive beneficial effect when used in combination with penicillin

These data give no indication as to the mechanism of the protective effect of generalized hypothermia, which depends both on the relative alteration of the pathogenicity of the organism and the resistance of the host Hypothermia may prolong the *in vivo* lag phase of bacterial growth as it does *in vitro* and thereby protect the host This would account for

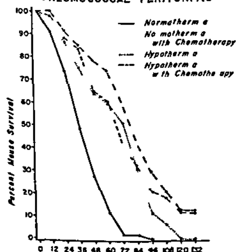


Fig 3 Comparison of Animal Survival Following Use of Hypothermia and Penicillin Both in Combination and Alone

the ineffectiveness of an antibiotic which primarily acts on multiplying organisms to improve survival in a hypothermic host where bacterial multiplication is markedly depressed Further studies are required to clarify the mode of action of hypothermia in this regard

SUMMARY

1 The therapeutic effect of prolonged generalized hypothermia on the course of pneumococcal peritonitis in white mice has been demonstrated
2 A combination of hypothermia and penicillin therapy produces no difference than the use of either agent alone
3 Possible mechanisms of the therapeutic effect of hypothermia in infection have been briefly discussed

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EFFECTS OF HYPOTHERMIA ON EXPERIMENTAL INTRACUTANEOUS PNEUMOCOCCAL INFECTION IN RABBITS*

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Patients in whom hypothermia was employed in association with cardiovascular operations have been noted to have a slight but significantly greater incidence of wound complications than when operation was performed at normal temperatures This problem has been generally attributed to inaccurate hemostasis during the period of relative avascularity associated with the hypotension of hypothermia The studies of Large and Heinbecker[3] showing a greater incidence of wound infection following local refrigeration and those of Bruneau and Heinbecker demonstrating acceleration of localized infection by refrigeration of the involved part upon rewarming suggest that increased susceptibility to infection may be a factor in the development of wound complications following the use of hypothermia

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This possibility became even more apparent to us in 1953 after observing the development of a fulminating pneumonia with septicemia following the emergency surgical repair of a vascular lesion under hypothermia in a patient who had a relatively minor chronic pulmonary infection. The latter observation also questioned the use of hypothermia in the treatment of systemic infections as previously suggested.[1,3]

This problem was investigated in the laboratory using a hemolytic staphylococcal infection in the dog. A severe septicemia was produced in the animals by injecting the bacteria intravenously and death occurred within 7 days in 20% of the normothermic animals and 90% of those cooled to 32° for 8 hours. These studies appeared to indicate that under these conditions hypothermia had an adverse affect upon infection and these preliminary results were reported at the Baylor University College of Medicine program for the Society of University Surgeons in April 1955. Subsequently, as these studies were being extended, the lyophilized source of staphlococci was inadvertently destroyed and the previous results could not be duplicated using another source of organisms; consequently these data were discarded and not published, although they suggested the possibility that hypothermia increased host susceptibility to infection.

Balch and associates[1] in 1955 were able to extend the life of rats with experimental peritonitis from 16 to 18 hours by employing continuous hypothermia and in 1956 Eiseman and associates[4] by employing prolonged (24 hours) and profound (19°C.) hypothermia were able to extend the life of mice for several hours with type III pneumococcal peritonitis; however, the ultimate survival rate at the end of 96 hours was similar in the cooled and normothermic animals. Frank and associates[5] in 1956 published a group of experimental results demonstrating that animals surviving hemorrhagic shock of 2 hours' duration die when injected with a dose of E. coli ordinarily well tolerated by the normal dog. In the experiment of these investigators animals subjected to hemorrhagic shock while hypothermic survived the intravenous injection of E. coli either during shock or after the animals were warmed to normal temperatures. Continuous or prolonged hypothermia in the first two experiments could possibly have both a bacteriostatic and bacteriocidal action on the organisms inoculated into the animals consequently altering the course of the infection. The factors involved in protecting the animals in the third experiment are not clear; however, in our experience hypothermia normally lowers the dog's blood pressure and to produce shock the removal of less blood is required in the hypothermic animal than in the normothermic animal. It is suspected that the results obtained by these investigators are in some way related to this factor.

To study the effects of hypothermia on infection, we felt that an experiment should be designed to test the influence of temporary cooling of moderate degree on survival of animals infected with virulent and avirulent organisms and a review of the literature revealed the work of Muschenheim, Duerschner, Hardy and Stoll[9] published in 1943 demonstrating the effects of hypothermia on rabbit pneumococcal infection. These investigators took advantage of the observations of Goodner on the manifestations of infection following intradermal inoculations of this organism in rabbits.[6,7]

He had shown that rabbits inoculated in this manner developed a local inflammatory lesion and if the strain of pneumococcus was sufficiently virulent bacteremia and death occurred in the majority of animals Certain strains which were nonlethal for rabbits were nevertheless capable of producing a local skin lesion The addition of pneumococcus autolysates increased the virulence of the organisms evident by more severe skin lesions and a greater incidence of bacteremia The previously mentioned investigators demonstrated in a small number of rabbits that hypothermia did not alter the lethal course of infection when virulent pneumococci were intradermally injected however when avirulent organisms were injected that ordinarily produce only a local lesion septicemia and death occurred in most instances within 48 hours Whereas the previous experiments appeared to be testing the effects of hypothermia upon bacterial growth the latter appeared to be testing the effects of hypothermia upon the resistance of the host and since it was testing the abnormalities in which we were interested it seemed advisable to repeat this work in view of the small number of rabbits involved Since the bacteriologic experimental preparation was ideally suited for our purposes it was adopted and studied more extensively

METHOD

Bacteria Supplies of avirulent pneumococcus Type III (American Type and Culture Strain 6303) obtained from a biologic outlet and an untyped virulent strain of pneumococcus obtained from a case of pneumococcal meningitis were lyophilized and retained as a bacterial source for all experiments A lyophilized sample of the organisms was reconstituted and cultured in media consisting of brain heart infusion and supplements of 1% glucose and normal rabbit serum The virulence of the Type III pneumococcus was adjusted to kill mice but not rabbits by injecting the organisms intraperitoneally into mice until it caused 100% fatality in these animals but remained nonlethal when injected into rabbits The virulence of the virulent organisms was adjusted by passage through mice until a significant mortality was obtained

In all infection experiments 0 2 cc (11 to 15 million viable organisms as determined by serial pour plate dilution) of an 18 hour culture of the appropriate organism was injected intradermally In the hypothermia group inoculation was performed immediately before induction of anesthesia

Animals Adult albino rabbits of both sexes weighing from 1 5 to 2 5 kg were obtained individually caged and observed for 5 days prior to the onset of the experiments to ascertain freedom from naturally occurring disease

Hypothermia The animals were anesthetized with phenobarbital 12 0 mg /100 gm body weight by intraperitoneal injection The animals were cooled to 31°C in approximately 30 minutes and maintained at this level for 8 hours using a cooling blanket At the end of 8 hours the animals were rewarmed using the same equipment Rectal temperatures were obtained constantly using an indlying standard laboratory thermometer and when normothermic temperatures were obtained the animals were returned to their individual cages

Observations. Blood cultures were obtained from the marginal ear vein at the onset of the experiment, daily, and at the time of death The rectal temperature, local lesion, and general condition of the animals were observed for 5 days These observations were performed in 116 animals divided into groups in the following manner (1) anesthesia alone, 10, (2) anesthesia and hypothermia, 10, (3) avirulent pneumococcus alone, 15, (4) avirulent pneumococcus and anesthesia, 10, (5) avirulent pneumococcus, anesthesia and hypothermia, 23, (6) avirulent pneumococcus, anesthesia, hypothermia and procaine penicillin® 200,000 units administered intramuscularly 12 hours before the experiment and at the time of anesthesia induction, 10, (7) virulent pneumococcus alone, 10, (8) virulent pneumococcus and anesthesia, 10, (9) virulent pneumococcus, anesthesia and hypothermia, 10, (10) virulent pneumococcus, anesthesia, hypothermia and penicillin as previously described, 8 rabbits

RESULTS

Anesthesia alone or anesthesia and hypothermia. All animals treated by anesthesia alone or by anesthesia and hypothermia survived the experiment without showing signs of illness

Virulent pneumococci. Severe skin reactions occurred in the 10 animals in which virulent organisms were injected intracutaneously as well as in the 10 animals injected with virulent pneumococci and subjected to anesthesia A febrile response was obtained in all of these animals and 40% of each group died approximately 48 hours after injection with positive blood cultures for pneumococci Pneumococci were cultured in the blood of the survivors at the end of 24 hours, however, subsequent cultures were negative All animals inoculated with virulent organisms and subjected to anesthesia and hypothermia died within 24 hours and the blood was positive for pneumococci in 6 of these animals In contrast to the violent skin reactions observed in the uncooled groups, only mild skin reactions were observed in the group subjected to hypothermia Death occurred in only 1 of the 8 rabbits injected with penicillin and subjected to virulent bacteria, anesthesia, and hypothermia This death occurred during the first 24 hours and was associated with pneumococcal bacteremia

Avirulent pneumococci. Mild skin reactions occurred in all animals injected with avirulent pneumococci and the 25 control animals into which this organism had been injected survived without bacteremia or a febrile response although 10 of them were anesthetized Death associated with pneumococcal bacteremia occurred within 72 hours in 16 of the 23 rabbits injected with avirulent organisms and subjected to anesthesia and hypothermia Bacteremia did not occur in the 7 surviving animals All animals (10) injected with avirulent pneumococci and protected with penicillin survived without bacteremia when subjected to anesthesia and hypothermia

DISCUSSION

The results obtained in this study which are statistically significant confirm those obtained by Muschenheim and associates[9] and demonstrate that generalized hypothermia of moderate degree does not protect the rabbit from the lethal effects of the intracutaneous injection of virulent pneumococci Moreover, it would appear that the virulence of this organism was

enhanced since a greater number of cooled animals died in a shorter period of time than the control animals injected with virulent organisms The conversion of a mild benign infection observed in 25 control animals injected with avirulent pneumococci into a lethal infection in 16 of 23 such animals subjected to hypothermia would appear to indicate that general hypothermia has an adverse effect upon pneumococcal infection in rabbits Although the factors responsible for these adverse effects are not apparent, the protection obtained by employing the appropriate anti bacterial agent is considered highly significant Survival in the control animals and those treated with penicillin indicates that infection was the predominant factor causing death in these animals The mechanism by which the virulence of infection was increased by hypothermia is not clear, but it would appear that cooling does not alter the bacteriostatic and bacteriocidal action of penicillin

SUMMARY

An increased incidence of wound infection has been noted in patients subjected to hypothermia during cardiovascular operations Although in accurate hemostasis during the hypotension of hypothermia may have been the responsible factor, certain evidence suggests that hypothermia increases host susceptibility to infection and questions the use of generalized hypo thermia in the treatment of systemic infection

Previous investigators recently have demonstrated that survival in rats with experimental peritonitis may be extended for a few hours by employ ing prolonged or continuous hypothermia The purpose of this experiment was to repeat and extend a study previously reported in which the effects of moderate hypothermia on virulent and avirulent intracutaneous pneumo coccal infection in rabbits were observed The results of the present study confirm those previously reported and demonstrate that hypothermia in creases the mortality rate in rabbits with a virulent infection and converts a benign avirulent pneumococcal infection into an infection that caused bacteremia and death in 69 per cent of the animals cooled Penicillin injected before and during the period of cooling prevented death in the majority of animals demonstrating that cooling does alter the antibacterial action of this antibacterial agent

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HOSPITAL INFECTIONS DUE TO ANTIBIOTIC-RESISTANT STAPHYLOCOCCI*

Bacteriologic and Clinical Experience and Methods of Control

Kenneth M Schreck, H Taylor Caswell, Elsie R. Carrington, Norman Learner, Howard H Steel, R Robert Tyson, and William H Wright

The past decade has witnessed a world wide increase in hospital acquired infections due to antibiotic resistant Staphylococcus aureus Today this organism represents the chief infectious agent with which the hospitals of this nation are confronted Like one of the first reports on this subject by Colbeck,[1] many papers have described epidemics confined to maternity units or other single departments of a hospital The present report, however, describes the magnitude of one year's experience with these infections occurring in *all* departments of a large general hospital

METHOD

Early in 1956 when it was apparent that Temple University Hospital was experiencing an unusual number of staphylococcal infections, a committee of representatives from the Department of Microbiology and the major clinical departments was appointed to study the problem and advise on methods of control When a coagulase positive *Staphylococcus aureus* was cultured from a patient, the committee determined if the patient was actually infected and, if so, classified the infection

All specimens for bacteriologic study were cultured aerobically and anaerobically on blood agar plates Specimens from the anterior nares of preoperative patients were obtained with sterile cotton swabs which were delivered to the laboratory in sterile nutrient broth Nasal swabs from personnel were inoculated directly onto blood agar plates

The coagulase test routinely used by our bacteriology laboratory was performed on all *Staphylococcus aureus* and *albus* cultured This report is concerned only with coagulase positive staphylococci All such organisms were typed with the 25 different bacteriophages kindly supplied by Dr John E Blair, of New York

*From Temple University Hospital and School of Medicine Philadelphia Pa, with the technical assistance of Dr T G Anderson A Troyanosky H Torop D Chang P Gage, R. Marganda B Jacobs A Krouse and T Henry

Eighty-seven patients (Table 1) who had no previous contact with the hospital, its personnel or discharged patients, were hospitalized during the 12 month period for treatment of established staphylococcal infections. It was surprising to find that the infections of 16% of these patients were caused by type 42B/52/81. This of course indicates that the organism did exist outside the confines of the hospital.

Nasal cultures obtained from 610 hospital personnel revealed 265 carriers of coagulase positive *Staphylococcus aureus*. However, only 11 of these were carrying the "epidemic" type. (Table 1)

It was soon evident that our personnel were having an unusual number of cutaneous infections. Personnel frequently treat themselves and remain on duty, therefore, these infections have to be sought out. During the year, 99 of our personnel had a total of 137 known staphylococcal infections, 65% of which were on the upper extremity or face, the areas most accessible to the patient; 80% of the total personnel infections were caused by type 42B/52/81. It should be noted that only 5 of the 11 nasal carriers of the "epidemic" type later suffered active infection and not all of the actively infected patients or personnel became nasal carriers.

The epidemic strain was not found in any of the spot checks of the inanimate objects. The hexachlorophene soap used in our surgical scrubs was found to be sterile as received from the manufacturer; however, as dispensed in surgery, this soap in all of its containers was contaminated with a viable coliform organism.

DISCUSSION

It appears that at about the time we became aware of our increase in patient infections other institutions were experiencing the same problem. The "epidemic" strain of *Staphylococcus aureus* is now known to be present in at least 12 of the United States, Germany, Australia (where designated type 80) and Canada (where called type 81).[2]

Methods to control this organism must be predicated on a firm knowledge of the epidemiology of staphylococcal infections. Although much has been written on this topic, it must be realized that most of the information is still speculative and that the true epidemiology of these infections is still unknown. We still do not really know how a staphylococcus is transmitted to a patient, what role the healthy nasal carrier plays in dissemination, or whether an organism in an airborne droplet nucleus has the same virulence as an organism in a draining abscess. Until these and other questions are answered, all methods of control are empiric rather than definitive.

To establish our methods of control, we first reappraised all procedures, corrected any breaches, and re-emphasized good surgical aseptic technique. Because of the coliform contamination of our hexachlorophene soap, our surgical scrub technique was changed to a 10 minute scrub with white soap and water followed by a 70% alcohol dip. It was advised that the operative site of the patients' skin be prepared with a soap and water scrub, followed by an ether rinse, followed by a liberal 70% alcohol rinse.

Penicillin as a 10,000 unit/ml. solution was instilled in surgical wounds at the time of closure just after the fascia had been approximated. The rationale of this procedure was to provide a high concentration of penicillin

to kill the few organisms that might have contaminated the wounds during surgery The results of this practice were extremely hard to evaluate

Patients with staphylococcal infections were isolated as well as possible Their linen was treated as contaminated linen The patients were treated conservatively with incision and adequate drainage Effective antibiotics were reserved for patients who were severely ill had extensive furunculosis or had pneumonia The use of prophylactic antibiotics in the postoperative period was discouraged

Personnel with active infection were removed from duty treated conservatively and only occasionally treated with antibiotics They were not returned to duty until all drainage had stopped We agree with Barber and Burston[3] that infected personnel play a major role while healthy nasal carriers play an insignificant part in the transmission of staphylococci to patients This impression is based on the large number of personnel with infections caused by type 42B/52/81 in contrast to the small number who were nasal carriers and also on several instances in which areas of the hospital experienced their first patient infections shortly after infected personnel were found on duty

Healthy nasal carriers were not removed from duty nor were they treated with antibiotics

Measures were instituted to improve the general housekeeping procedures throughout the hospital

Having instituted the control measures briefly described above we noted a reduction in the incidence of infections from a peak in March 1956 to a level we consider acceptable It is impossible to say which if any of the measures was responsible for the reduction Obviously in a situation such as this one must invoke any measure of control considered useful therefore many changes have to be made at once and a controlled study is thus precluded Ideally of course complete eradication of the organism from the hospital should be sought but the institution which has been able to do this is exceptional

SUMMARY

*A 12 month study in which 323 patients acquired staphylococcal infec*tions during hospitalization is described The epidemic type 42B/52/81 *Staphylococcus aureus,* which now has world wide distribution was the main offending organism The characteristics of this organism and our empiric methods of control are discussed

It appears that hospital personnel with active infection are more important in transmitting this organism to patients than are healthy nasal carriers among the personnel

Type 42B/52/81 staphylococcus also exists outside the hospital since 16 per cent of the patients hospitalized for treatment of existing staphylococcus infections were infected with this organism

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ETHYLENE OXIDE STERILIZATION—A NEW METHOD*

J H Davis, J S Wolkoff, and J R Leonards

There has long been a need for a simple, safe, effective method of sterilizing surgical supplies which are not amenable to heat sterilization Such items include the various "scopes" with their complex lens systems, the modern plastic materials used in surgery, certain suture materials, and delicate metallic instruments subject to corrosion with heat sterilization Ethylene oxide vapor provides a method of sterilization which will not damage these special surgical supplies

METHOD

Ethylene oxide, an epoxy compound, consists of a three membered ring two carbon and one oxygen atom It boils at 10 8°C and freezes at −111 3°C It is a gas at ordinary temperatures and pressures but is easily liquefied It is soluble in water, alcohol and ether It is highly flammable, and the vapors form an explosive mixture with air in all proportions from 3% to 100% by volume When mixed with more than seven times its volume of carbon dioxide the explosive danger is eliminated Carboxide, a commercial product consisting of 10% ethylene oxide and 90% carbon dioxide, has been produced This mixture is available in tanks with the gas stored at a pressure of about 800 pounds per square inch and requires an expansion chamber on release of the gas to prevent fractionation and to reduce the pressure to about 80 pounds per square inch [1]

The addition of 20% ethylene oxide to 80% Genetron† and packaging the material in small cartridges (Steribulb) has made available the highly potent sterilizing action of this compound in an easy to handle and safe unit The Ben Venue Laboratories have also built a small portable gas chamber which utilizes the Steribulb (Fig 1) All of our studies were carried out in this unit

The effective sterilizing potential of ethylene oxide was first tested on metallic instruments using stock cultures Hemostats with a box lock, a cystoscope, ring clamps and other small laboratory equipment with joints ratchets and the like were studied Cultures (24 hour broth) of *Escherichia coli*, *Pseudomonas aeruginosa* *Staphylococcus aureus* *Bacillus subtilis*, and *Mycobacterium tuberculosis* were utilized

The instruments were contaminated directly placed in the gas chamber and a bulb was fired After 60 minutes the sterilizer was opened and the instruments removed under aseptic conditions Cultures were taken from the instrument and placed in tryptose phosphate broth thioglycollate broth and on a blood agar plate Specialized media such as that required for the growth of *Mycobacterium tuberculosis* was used when indicated Approximately 50 such tests were carried out and in all instances the cultures failed to show any growth

The type of organism which could be killed was next evaluated The different organisms tested are listed in Table 1

†Steribulbs—Ben Venue Laboratories—Bedford Ohio

*From the Departments of Surgery and Biochemistry Western Reserve University School of Medicine and the University Hospitals of Cleveland

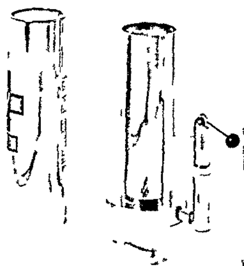


Fig 1 The Ben Venue Sterilizer From left to right 1) The outer jacket with adsorbent filter in the top 2) The sterilizing chamber with its attached piercing assembly The steribulb is inserted by loosening the knurled knob in the center of the piercing assembly Foreground the steribulb

Table 1 Organisms Tested

Non Spore Forming Bacteria	Spore Forming Bacilli	Yeasts
Pseudomonas aeruginosa	*Bacillus anthracis*	*Candida*
Staphylococcus aureus	*Bacillus globigii*	
Micrococcus tetragenus	*Bacillus megatherium*	
Escherichia coli	*Bacillus subtilis*	
Klebsiella pneumoniae	*Clostridium botulinum*	
Proteus vulgaris	*Clostridium perfringens*	
Salmonella typhosa	*Clostridium tetani*	
Myobacterium tuberculosis		

The final step in this study was to evaluate the effect of ethylene oxide on the particular materials which cannot be sterilized by boiling or auto claving Most of these materials require soaking for 18 to 24 hours in a bactericidal solution or vapor sterilization by formalin Both of these methods are unsatisfactory especially for spores and, in addition, are very time consuming The materials tested are listed in Table 2 There was no evidence of damage to any of these materials after sterilization by this method

Table 2 Instruments and Equipment Tested

Ampoules of surgical catgut	*Hemostats*
Catheters—plastic	Needles
Catheters—rubber	Neurosurgical burrs and perforators
Chemo pallidectomy needles	Reese Dermatome tape
Clinical thermometers	Scalpel blades
Cystoscopes	Scalpel handles
Dermatome (Reese) blades	Scissors
Dilators	Sutures—silk
Forceps	Syringes
Heart lung catheters	

The operating room supervisor found the method valuable for reclaiming unbroken glass tubes of surgical catgut *Since prolonged soaking of* these is necessary once they are removed from their plastic container, they are a nuisance to reclaim At present they are wrapped and sterilized with ethylene oxide at a considerable saving of time

DISCUSSION

The need for a safe effective sterilizing agent other than heat has long been known Ethylene oxide gas is an effective compound and has been used in industry for at least 10 years It has not been employed in medicine primarily because of its explosiveness The recent development of 20% ethylene oxide mixed with 80% Genetron has provided a safe method of handling the material even in the presence of open flame

The gas having a similar toxicity to ammonia is relatively nontoxic in the amounts used It is odorless in ordinary concentrations but will cause smarting of the eyes headaches nausea and vomiting when over dosage is present One hundred parts per million is a safe level for prolonged exposure The quantity of ethylene oxide contained in the Steribulb is well below the maximum allowable for 8 hours continuous exposure in a small unventilated room The sterilizing chamber used in these studies has an adsorbent filter through which the gas is removed and allowed to slowly escape into the room air Accumulation in the body does not occur so that chronic poisoning is not a problem

The exposure time necessary for complete sterilization would appear to be about one hour under the conditions of these experiments Temperature and moisture affect the speed of sterilization These tests were conducted at 65°F to 85°F and at average humidity By raising the temperature to 100°F to 140°F and using a relative humidity of 25% to 50% a shorter time for sterilization is required Ordinary working room temperature and humidity are perfectly satisfactory however since shortening the exposure time would appear to be of little practical value

SUMMARY AND CONCLUSIONS

1) Ethylene oxide gas has been shown to be an effective sterilizing agent and has been used in industry for the past 10 years

2) The high reactivity and difficulty in handling the material has been eliminated by the mixture of 20% ethylene oxide and 80% Genetron

3) A variety of instruments have been contaminated with many different pathogenic microorganisms and subjected to ethylene oxide vapor in a small portable chamber All of the materials tested were sterile at the end of one hour of exposure and none showed evidence of damage

4) The simplicity and safety of the equipment described would appear to make available a very effective sterilizing system for items which cannot withstand heat sterilization

5) The use of such a system may give manufacturers of surgical equipment a wider variety of materials with which to work and thereby improve the final product

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CLORPACTIN A SURGICAL ADJUNCT*

Antimicrobial and Tumorcidal Action

MARVIN L GLIEDMAN ROALD N GRANT BETTY L VESTAL
CHARLES E ROGERS AND KARL E KARLSON

Clorpactin † a form of monoxychlorosene has been under investigation for the past year both experimentally and clinically as a lavage fluid for use during surgery This communication describes experimental studies on the antimicrobial and tumorcidal properties of this organic hypochlorous acid derivative

METHOD

Antimicrobial Action Nonfasting adult mongrel dogs were used The entire colon was exposed at laparotomy A 1000 cc saline colonic irrigation was introduced into the cecum through a 13 or 15 gauge needle attached to a sterile intravenous set The irrigation fluid was evacuated through a No 38 French colonic tube inserted into the rectosigmoid either through a purse string suture or by passing it via the anus intraluminally to this level Umbilical tapes were utilized to isolate the colon from the ileum to the rectum The saline lavage mechanically cleansed the colon The saline colonic irrigation was followed by a 0 4% Clorpactin WCS 90 wash A long longitudinal colotomy was performed on the colons irrigated with Clorpactin WCS 90 This colotomy was closed and re examined 6 to 8 weeks later Three hundred cc of Clorpactin WCS 90 was left in the peritoneal cavity On sacrifice 6 to 8 weeks later the peritoneal surface and mucosa were studied for effects that might be attributable to this agent

Specimens were taken of the wash throughout the procedure and were cultured for bacteria yeasts and molds Clorpactin WCS 90 was inactivated immediately after its exit from the colonic tube with an equal volume of 0 1N sodium thiosulfate[1] in two experiments

Trypticase soy agar and trypticase dextrose extract was used for bacterial cultures Sabouraud s dextrose agar for the mold cultures and Wort agar for the yeast cultures Bacterial plates were incubated for 24 hours at 35 to 37°C and mold and yeast plates at 28 to 30°C for 5 to 7 days At the end of the incubation periods the developed colonies were counted These counts were expressed as counts per cubic centimeter of colonic wash fluid

Tumorcidal Action *In vivo* studies Solid Walker rat carcinosarcoma 256 was emulsified in a commercial kitchen homogenizer and diluted in the control group with 0 9% saline Clorpactin XCB a purified higher potency Clorpactin in a 0 4% dilution in saline was used as a diluent in the study group The first group of Sherman strain rats were inoculated

†Clorpactin WCS 90 and Clorpactin XCB manufactured and supplied by the Guardian Chemical Corp Long Island City New York

*From the Department of Surgery Surgical Research Laboratory U S Naval Hospital St Albans New York and the Department of Surgery of the State University of New York College of Medicine Brooklyn New York Aided by a contract between the Office of Naval Research Department of the Navy and the State University of New York

with 1 cc. of the tumor emulsion containing 50,000 cells (by hemocytometer count) plus 4 cc. of either the 0.9% saline or 0.4% Clorpactin XCB in saline. The cell suspensions were allowed a contact time of 3 minutes before inoculation into the peritoneal cavity. The Clorpactin XCB study group was inoculated first to insure that if the short delay (15 minutes) between inoculating the study and control groups decreased the virulence of tumor cell suspensions, the less viable cells would be inoculated into the control animals. The animals were autopsied on death; those that survived one month were sacrificed and autopsied.

The experiment with Walker rat carcinosarcoma 256 was repeated with a cell inoculation of 100,000 cells. A control group of inactivated Clorpactin XCB† as well as a saline control group were utilized in this study.

Studies were also carried out on albino mice using Sarcoma 180, an ascites producing tumor. Two tenths of 1 cc. of cell suspension (over 100,000 cells) with either 0.8 cc. of isotonic saline or 0.8 cc. of 0.5% Clorpactin XCB in saline was inoculated. The technique was similar to the Walker rat carcinosarcoma 256 groups. Animals that succumbed in the first 24 hours were excluded from the study.

***In vitro* studies.** Using a supravital stain technique[2] (neutral red-Janus green) the conversion to nonviable cells was studied.

Two tenths of a cubic centimeter of sarcoma 180 ascites fluid containing approximately 200,000 cells was diluted with 0.8 cc. of 0.9% saline, 10% formalin, 0.5% Clorpactin XCB or 0.5% inactivated Clorpactin XCB. Immediately after mixing, the tumor cell suspension was placed on a cover slip and then upon a microscope slide covered by a dried film of the Janus green-neutral red mixture. A representative field of tumor cells was rapidly brought into low power focus and at 30 second intervals the percentage of nonviable cells was recorded. Nonviable cells were those whose nucleus became brightly stained pink from the neutral red; the viable cells remained unstained except for cytoplasmic bodies stained with Janus green.

The observer tabulating the percentage of nonviable cells was not aware of the diluent solution used to prepare the tumor suspension under study. The tabulating of nonviable cells continued until 300 seconds had passed.

RESULTS

Antimicrobial Action. The results of the bacterial, yeast, and mold counts are in Table 1 and Figure 1. Two hundred cubic centimeters of Clorpactin were sufficient to reduce the microbial counts to near zero. In only one instance (out of 24 separate culture specimens) was an organism cultured from the wash solution after more than 200 cc. had passed through the colon (dog #21, one bacterial colony/cc.) The Clorpactin effected the same decrease in yeast and mold counts as in bacterial counts.

There were no apparent untoward gross or microscopic effects to normal and healing tissues washed with 0.4% Clorpactin WCS 90.

Tumorcidal Action. The results of the tumor inoculation studies are in Table 2. Small volumes (4.0 and 0.8 cc.) of 0.4% and 0.5% Clorpactin XCB were quite effective in killing large concentrations of tumor cells.

†Inactivated Clorpactin XCB (rendered inactive by strong light and exposure to air for seven days), supplied by the Guardian Chemical Corp.

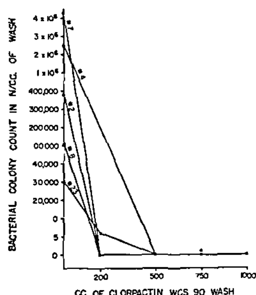


Fig 1 The fall in bacterial counts following Clorpactin WCS 90 colonic irrigation The zero colony count from 500 to 1000 cc represents culture data from animals #4 #18 and #23 although one bacterial colony/cc was cultured following 750 cc of irrigation (dog #23 see Table 1)

Table 1 Bacterial, Yeast, and Mold Counts after Clorpactin WCS 90 Washed Through Bowel (Colonies/cc of Wash Fluid)

Dog #		2	4	7	18	23
	B*	380 000	2 5 x 10⁶	4 3 x 10⁶	110 000	30 000
Starting Count	Y*	**		4 5 x 10⁶		
	M*	0		0		
	B	0		0	0	6
200 cc Clorpactin	Y	0		0	0	
	M	0		2	0	
	B	0	0	0	0	0
500 cc Clorpactin	Y	0		0	0	0
	M	0		0	0	0
	B				0	1
750 cc Clorpactin	Y				0	
	M				0	
	B		0		0	0
1000 cc Clorpactin	Y				0	0
	M				0	0
Remarks		Neutralized with 0 1 N Sodium Thiosulfate			Immediate plating	

*B—Bacteria Y—Yeasts M—Molds
**Growth present on yeast plates but not typically yeasts

In Figure 2 the results of the supravital staining are graphed Clorpactin XCB was effective in rendering the tumor cells nonviable The percentage of nonviable cells in 0 5% Clorpactin XCB was not statistically different from the percentage of nonviable cells in 10% formalin at similar time intervals

DISCUSSION

The adequacy of Clorpactin WCS 90 as a safe antimicrobial agent experimentally has prompted its use clinically as a lavage fluid for the

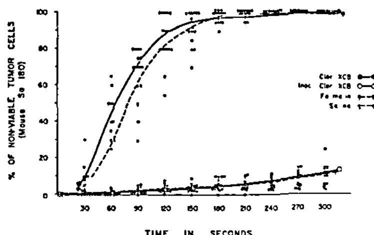


Fig 2 Percentage of cells rendered nonviable in various diluents Supravital staining with Janus green and neutral red Approximate cell count 200 000 The curves are drawn through the means of the points at each time interval

Table 2 In Vivo Inoculation of Tumor Suspension

TUMOR	CELL COUNT OF INOCULUM (APPROX)	DILUENT	NO OF ANIMALS	NO WITH TUMOR	PERCENTAGE WITH TUMOR
Sherman strain white rat	50 000	Saline (.9%)	8	7	88
Walker carcinosarcoma 256	cells	Clorpactin XCB (.4%)	5	0	0
Sherman strain white rat	100,000	Saline (.9%)	11	9	82
Walker carcinosarcoma 256	cells	Inactive Clorpactin XCB (.4%)	10	8	80
		Clorpactin XCB (.4%)	11	1*	9
Albino mouse sarcoma 180	100 000	Saline (.9%)	23	23	100
	cells	Clorpactin XCB (.5%)	13	0	0

*This animal was partially eaten and considerably decomposed when removed from the cage adequate gross or microscopic study was not possible Although no tumor was demonstrated in this animal, it is included in the positive group

peritoneal cavity, as a colonic irrigant preceding and during colotomies and as a wound irrigant The results have been gratifying

Organic debris will compete with the organism for Clorpactin, limiting the antimicrobial or tumorcidal effect For this reason, copious amounts of Clorpactin have been used clinically for lavage Inactivated Clorpactin is an organic compound (hydrocarbon derivative) which might be utilized as a bacterial nutrient While we noted no difficulties in leaving 300 cc of Clorpactin WCS 90 in the abdominal cavity of dogs, there are theoretical objections to leaving a possible microbial nutrient in the operative field The excess Clorpactin is therefore removed following lavage

Work previously published[3 4 5] has demonstrated the lethal effects of this agent on protozoa, viruses and acid fast bacilli after short contact periods This rapid, apparently universal, spectrum on unicellular organisms suggested the tumorcidal studies

One animal is listed as a tumor "take" in the animals inoculated with tumor cells and Clorpactin XCB (Table 2)

A supravital staining technique was employed on the suggestion of Dr Warren H Cole This technique showed that the sarcoma 180 cells in a small quantity of 0 5% Clorpactin XCB became rapidly nonviable The rapid death of sarcoma 180 cells (generally faster than 10% formalin)

slowed somewhat after 120 seconds suggesting perhaps that the available hypochlorous acid gas and OCl , the active ingredients in the Clorpactins had been rapidly used to kill cells and the remaining viable cells were acted upon by a relatively low concentration of remaining active ingredients in the cell Clorpactin XCB suspension The large volume of 0 5% Clorpactin XCB used clinically obviates this problem

The effect of Clorpactin XCB on tumor cells does not appear to be tumor specific Normal desquamated mesothelial cells and leucocytes have been noted to become nonviable in Clorpactin XCB

In the studies with Walker rat carcinosarcoma 156 cell suspensions clumps of tumor cells were seen in the hemocytometer chamber and in smears made from material used for inoculation, the emulsion was often so viscid that there was difficulty in drawing it through a #18 gauge needle into the syringe for inoculation Since the presence of clumps of tumor cells (approximately 20 cells) did not detract from the efficacy of the Clorpactin XCB it would seem that either the hypochlorous acid gas and OCl permeated the depths of the cell group or that the Clorpactin XCB destroyed the surface cells of the group and thus prevented those cells in the core of the cell clump from obtaining nutrition or from implanting on the peritoneum

We are at present utilizing 0 5% Clorpactin XCB routinely as a lavage fluid during cancer surgery The material is used in the wash basins and is during the course of surgery, used to irrigate the field At the conclusion of surgery the field is flooded with 0 5% Clorpactin XCB and lavaged for at least 3 minutes One thousand cubic centimeters of Clorpactin XCB is used in the lavage In over 20 patients we have noted no damage to intact tissue or toxic effects from this agent Wound healing grossly has not been deleteriously affected

SUMMARY

1 Two hundred cubic centimeters of 0 4% Clorpactin WCS 90 washed through a mechanically clean canine colon routinely reduced microbial counts to trace levels The Clorpactin WCS 90 caused the same decline in yeast and mold counts as in bacterial counts

2 Walker carcinosarcoma 256 and sarcoma 180 cells suspended in 0 4% and 0 5% Clorpactin XCB did not grow following inoculation into the peritoneal cavity of rats and mice

3 Supravital staining showed sarcoma 180 cells to be rapidly rendered nonviable in 0 5% Clorpactin XCB

4 Clorpactin WCS 90 and Clorpactin XCB have been used clinically and no injurious effects have been noted from the materials

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FURTHER STUDIES OF HUMAN ADRENAL VEIN BLOOD SECRETION OF ESTROGENS AND 17 KETOSTEROIDS*

JAMES D HARDY, VIRGINIA B WARD, M DON TURNER, AND LOIS P SAMPSON

In a previous communication we reported investigations concerning the rate of hydrocortisone secretion in man[2] The data were achieved by means of left adrenal vein blood samples collected over a timed interval at laparotomy It was found that under the stress of laparotomy the rate of hydrocortisone secretion may be on the order of 111 mg/24 hours, and under normal conditions, perhaps 34 mg/24 hours These estimations were based on plasma hydrocortisone content only It has been estimated that a 25% increment should be added for red cell hydrocortisone content

The purpose of the present communication is to report adrenal vein estrogen and 17 ketosteroid concentrations during operation, as compared with those in peripheral blood before, during, and following operation In a few instances it has been possible to obtain ovarian venous plasma estrogen determinations for comparison with levels in adrenal venous blood and in peripheral blood Finally, further measurements of rates of hydrocortisone secretion have, in general, further validated the values previously reported from our laboratory

METHOD

During the course of laparotomy performed for various procedures such as cholecystectomy or gastric resection the left central adrenal vein was exposed by dividing the gastrocolic omentum, incising the posterior parietal peritoneum along the inferior margin of the pancreas, and gently retracting this organ anteriorly and cephalad with a Deaver retractor A silk ligature was passed around the adrenal vein just at the point of juncture with the left renal vein, this ligature was temporarily tightened during aspiration of adrenal vein blood over a timed interval to prevent contamination of adrenal vein blood by renal vein blood Simultaneous peripheral vein sampling was performed as well as preoperative and postoperative peripheral blood sampling

The total estrogens determinations were performed by a modification of the method of Brown[1] The 17 ketosteroid determinations were performed by the method of Migeon[3] These exploratory measurements were performed by Bio Science Laboratories, Los Angeles California

RESULTS

Plasma Estrogen Levels *Adrenal vein plasma* estrogen levels were obtained in 10 patients The mean concentration was 8 0% micrograms

*From the Department of Surgery University of Mississippi Medical Center Jackson Mississippi Aided by Army Contract No DA-49 007 MD 627

(range, 3 2 to 12 4) The *peripheral plasma* estrogen concentrations were satisfactorily completed in few patients early in the study, however, in a complete and most careful study of 2 patients late in the experiments, the values were all less than 2 gamma before, during, and following surgery Further measurements of peripheral plasma estrogen levels are necessary before definitive conclusions can be drawn

In a comparison between the *ovarian vein plasma estrogen* concentrations *in 3 women below the age of 40 (average, 28 years) with 4 above the age* of 40 (average, 57 years), it was found that the average estrogen concentration in the younger women was 9 1 gamma (range 3 3 14 1) while that in the older group was 2 8 gamma (range 1 2 6 8) Nevertheless, it will be noted that the ranges did overlap to some extent, and additional measurements will be obtained There was less average difference between the adrenal plasma estrogen levels of these two groups of women (than between ovarian vein levels), and there was much more overlapping in individual values

Plasma 17-Ketosteroid Levels. There was an extremely consistent pattern in the 17 ketosteroid concentrations Whereas peripheral plasma levels generally fell within the normal range (about 40 120 *ug*/100 cc of plasma) —whether the blood was taken before, during or following surgery—the adrenal plasma concentrations lay above 150 *ug* (range, 157 377) Therefore, if it be assumed that substances reacting as "17 ketosteroids' consist largely of androgens or androgen derivatives, it will be clear that the adrenal gland does secrete sizeable amounts of the steroid compounds

In 3 of 5 women so studied, the plasma 17 ketosteroid levels of ovarian vein blood were above average peripheral plasma levels (194, 217, and 290) It is apparent that the ovarian secretion also contains substances which give the "17 ketosteroid' reaction Again, in no instance were peripheral plasma 17 ketosteroid values above generally accepted normal levels

DISCUSSION

In view of the well known role which androgens and estrogens play in the growth of hormone dependent tumors such as those of the breast and prostate, the findings reported herein re emphasize the roles which the ovaries and adrenal glands may play in the natural history of such tumors Both the adrenal cortex and the ovary secrete substances which react chemically as 'estrogens' or '17 ketosteroids' It would appear that these organs are capable of affording hormonal support for certain androgen or estrogen dependent tumors and that to abolish androgen and estrogen secretion these hormonal sources must be removed However, total adrenalectomy produces a physiologic state that is not to be taken lightly and recommendation of this operation for the management of tumors of the breast and prostate is not necessarily implied by us on the basis of the data reported herein

SUMMARY AND CONCLUSIONS

1 Plasma estrogen and 17 ketosteroid levels in peripheral, adrenal, and ovarian venous blood have been reported

2 The adrenal plasma estrogen and 17 ketosteroid levels sharply exceed those of peripheral blood

3 Previously reported rates of hydrocortisone secretion have been further substantiated in the course of the present study of estrogen and 17 keto steroid secretion rates

4 Both the ovary and the adrenal gland are capable of secreting significant amounts of both "estrogens" and "17 ketosteroids"

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THE SECRETION OF EPINEPHRINE, NOR EPINEPHRINE AND CORTICOSTEROIDS IN THE ADRENAL VENOUS BLOOD OF THE DOG FOLLOWING SINGLE AND REPEATED TRAUMA*

DAVID M HUME

It is known that increases in both adrenal medullary and adrenal cortical secretion usually follow injury The present study was undertaken in an effort to delineate the pattern of this secretory response following single and repeated operative trauma The adrenal secretion of 17 hydroxycorticosteroids, epinephrine, and nor epinephrine was measured directly in the resting state, and these values were compared with those seen following operative trauma under ether or nembutal anesthesia, and following anesthesia alone without operative trauma The continued ability of the adrenal medulla to secrete epinephrine and nor-epinephrine was checked periodically by the injection of nicotine intravenously The function of the adrenal cortex was likewise assessed by injections of ACTH

METHOD

The studies reported here were carried out on 30 mongrel dogs, weighing from 9 9 to 15 8 kg The adrenal vein was cannulated by the method of Hume and Nelson[1] permitting repeated collections of adrenal venous blood, and measurements of adrenal blood flow, in the conscious animal Samples were collected over 0 5 to 3 0 minute periods, depending on the rate of flow

The blood samples obtained were assayed for 17 hydroxycorticosteroids by the method of Nelson and Samuels[2] Measurements of epinephrine

*From the Surgical Research Laboratory Department of Surgery, Medical College of Virginia Richmond Supported in part by a grant in aid from the Commonwealth Fund, and in part by the United States Air Force under contract number AF 41(657) 169, monitored by the School of Aviation Medicine, USAF, Randolph Air Force Base, Texas

and nor epinephrine were carried out by the method of Weil Mall and Bone,[3] as modified by Aronow Nembutal anesthesia was given i venously in a dose of 30 mg /kg When ether anesthesia was empl the dog was induced with just enough pentothal to permit introdu of an intratracheal tube, after which anesthesia was maintained wit ether oxygen mixture, using a pneophore for forced respiration All v are recorded in micrograms secreted per minute from one adrenal (γ/r

The initial operation was the placement of the cannula and ad vein snare Samples were taken as soon as the cannula was in place, at other intervals during the operation Sampling was continued fr to 4 hours postoperatively, and daily thereafter from 1 to 12 days A se anesthesia was administered at some time during the convalescent pe After obtaining samples from 2 to 3 hours to note the effect of the thesia alone, the animal was suddenly traumatized by making a abdominal incision and withdrawing the bowels onto a laparotomy Further samples were taken to determine the effect of this second o tion upon adrenal secretion

Nicotine was administered intravenously in a dose of 0 1 mg /k test adrenal medullary reactivity It always produced a profound, th transient, effect, consisting of vomiting or retching, excitement, and, ra a brief and minor convulsion ACTH was administered intravenous a dose of 1 to 40 units to test adrenal cortical reactivity It was unac panied by any systemic effect

RESULTS

In Table 1 are recorded the mean corticoid and epinephrine v obtained during operation under nembutal or ether anesthesia an the first postoperative day It may be seen that whereas both grou animals show a similar corticoid response, there is a much greater ou of epinephrine in the ether group (10 5 times as great) Control values the 2nd to 12th day were similar in both groups and averaged 061 γ/ for epinephrine and 010 γ/min for nor epinephrine There is, there a relative increase in the proportion of epinephrine to nor epinephrin the ether dogs as well as an absolute increase of both substances

In Table 2 are recorded the effects of ether and nembutal anest alone and in combination with a second operation in the convales period It is apparent that whereas ether itself acts as a marked stim to epinephrine and, to a lesser extent, nor epinephrine production, n butal depresses this production below that seen in the resting state

Table 1 Mean Values During Operation and First Postoperative D

		NO OF DOGS	17 OH CORTICOIDS γ/MIN	EPINEPHRINE γ/MIN	NOR EI γ/MIN
Day of operation	Ether dogs	10	14.2	558	095
	Nembutal dogs	20	12 3	052	035
1st post op day	Ether dogs	4	1.5	034	009
	Nembutal dogs	15	3 8	076	037

Table 2 Effects of Anesthesia and Operation in Convalescent Period

	MEAN CONTROL VALUES γ/MIN EPI	N E	MEAN MAXIMAL RESPONSE γ/MIN EPI	N E	NO OF DOGS
Ether anesthesia alone	076	039	427	137	5
Nembutal anesthesia alone	017	036	017	019	4
Ether anesthesia plus operation	.202	073	1 695	.319	3
Nembutal anesthesia plus operation	000	009	012	081	1

infliction of operative trauma on the dog under nembutal anesthesia produced no significant effect on epinephrine production, although there was some increase in nor epinephrine production There was an imme diate marked increase in corticosteroid output (See Fig 1) By contrast, trauma to the dog under ether anesthesia produced a tremendous and prolonged increase in epinephrine and nor epinephrine production (See Figs 2 & 3) This was true even if the animal was in shock, with decreased adrenal blood flow (See Fig 3) There was again an increase in cortico steroid production as well unless it was already nearly maximal (Fig 2), or unless the adrenal blood flow fell to very low levels (Fig 3) With moderate levels of shock there is often an increased corticosteroid output (Hume and Nelson[1])

In all instances the response to nicotine was instantaneous, marked, and very transient The mean control value for epinephrine was 060 γ/min which rose to 9 262 γ/min 2 minutes after injection, and fell to 054 γ/min 10 to 15 minutes after injection A transient increase in corticosteroid output was also noted

The injection of ACTH was followed by a rapid increase in cortico steroid secretion, except when it was already maximal during operative trauma No effect on epinephrine or nor epinephrine secretion was noted

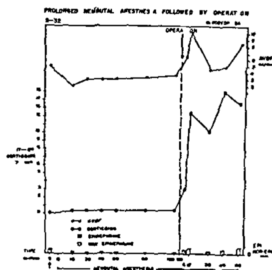


Fig 1 Adrenal blood flow 17 hydroxycor ticosteroid epinephrine and nor epineph rine secretion are charted The dog was maintained under nembutal anesthesia for 2 hours at which time an operative trauma was carried out A marked increase in cor ticosteroid secretion but no increase in epinephrine and only a modest increase in nor epinephrine secretion followed

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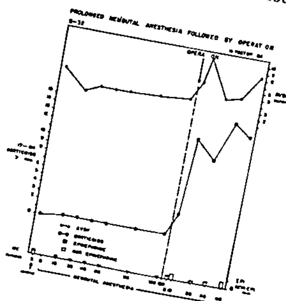


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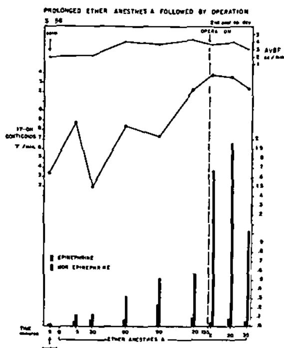


Fig 2 The dog was maintained under ether anesthesia for 2 hours during which time there was a gradual but progressive increase in epinephrine nor epinephrine and corticosteroid secretion Operative trauma produced an additional sharp rise in epinephrine secretion, while the rise in corticosteroid secretion was not marked since it was already nearly maximal for this animal

These results suggest that ether anesthesia is itself a trauma, accompanied by increased adrenal activity, both medullary and cortical, and that operative trauma under this anesthesia is a further stimulus to adrenal secretion Nembutal anesthesia, on the other hand, suppresses adrenal medullary activity, probably at a hypothalamic level, even when accompanied by operative trauma Whether either of these circumstances may offer special advantages to the operative patient is a matter for further experiment and speculation

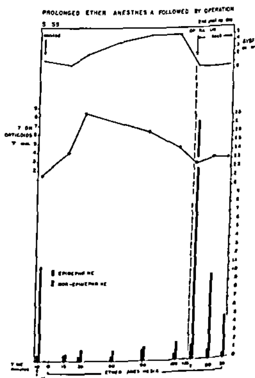


Fig 3 The circumstances were the same as shown in Fig 2, except that the dog went into shock with the operative trauma A very marked increase in epinephrine and nor epinephrine output followed The failure of the corticosteroid output to rise with this trauma may have been due to the depth of shock and degree of depression of adrenal blood flow

CONCLUSIONS

1. Ether anesthesia alone produced an increased adrenal secretion of epinephrine, nor-epinephrine, and 17 OH corticosteroid over that seen in the resting conscious dog.

2. Operative trauma under ether anesthesia produced an additional increase in epinephrine and nor-epinephrine secretion, even in the presence of profound shock. Corticosteroid secretion was increased also, except when the shock was so severe as to lead to marked decreases in adrenal blood flood.

3 Nembutal anesthesia alone depressed the secretion of epinephrine and nor epinephrine.

4 Operative trauma under nembutal anesthesia was accompanied by marked increases in coticosteroid output, but no significant increase in epinephrine, and only slight increase in nor epinephrine, secretion.

5 An increased adrenal cortical secretion began within 3 minutes after injury. Increased medullary secretion, when present, was also very rapid in onset, beginning within 2 minutes after injury.

6 A second operation in the convalescent period produced an adrenal response which was equal to or greater than that seen with the first operation.

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STEROID METABOLISM IN INFANTS EFFECT OF SURGERY ON PLASMA 17 21 HYDROXYCORTICOSTEROID LEVELS INTERIM REPORT*

JESSE L WOFFORD, JAMES D HARDY, M D TURNER AND WILLIAM A NEELY

The function of the adrenal cortex in infants has not often been studied by determination of hydrocortisone levels in plasma And more particularly, information regarding the steroid metabolism of the infant adrenal cortex following surgical trauma is still fragmentary We have measured the plasma levels of 17 21 hydroxycorticosteroids before and following operation in 11 infants and young children The results obtained indicate that adrenocortical response to stress in infants is relatively active, and that adrenocortical replacement therapy is not indicated as a routine measure following surgery in such patients

METHOD

Eleven children below the age of 2 years underwent elective surgical procedures using general anesthesia None of the subjects was debilitated and selection for study was based solely upon age The surgical operations varied in magnitude from unilateral hernioplasty to pulmonary lobectomy Ages of the subjects ranged from 1 month to 17 months but 9 were less than 4 months of age

Three samples of venous blood were drawn from each patient The control sample was taken immediately before operation prior to the onset of anesthesia, further samples were obtained at 4 hours and 24 hours following operation Each blood specimen was heparinized and centrifuged The plasma levels of free and conjugated hydrocortisone were determined by a microtechnique adopted from the work of Nelson and Samuels[1] and Bongiovanni [2]

RESULTS

Free 17 21 hydroxycorticosteroids (essentially hydrocortisone) in the control specimen averaged 2 73 ug/100 ml of plasma while conjugated forms averaged 1 27 ug/100 ml of plasma Four hours after surgery they were 5 36 and 2 57 ug/100 ml respectively The concentration of 24 hour levels of both free and conjugated steroid forms were approximately the same as preoperative levels The absolute concentrations of the corticoids in plasma were low when compared with those of adults we have studied but they were in agreement with values for infants obtained by other laboratories

DISCUSSION

Adrenocortical Insufficiency In Infants. Adrenocortical insufficiency occurring in infancy is not unknown For example, serious impairment of certain metabolic functions of the adrenal cortex is not uncommon in the adrenogenital syndrome in fact adrenocortical insufficiency for electrolyte metabolism may result in death The masculinization is due to excess

*From the Department of Surgery University of Mississippi Medical Center Jackson Mississippi Supported by Army Contract No DA 49 007 MD 627

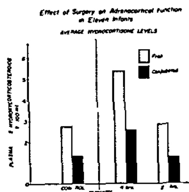


Fig 1

androgen production, whereas it is the hydroxycorticosteroids that are necessary for life Salt loss[3] and periodic hypoglycemia[4] are evidence that essential cortical hormone production in the adrenogenital syndrome is hampered by the diffuse hyperplasia of the adrenal gland and may result in circulatory collapse Failure to recognize the potential adrenal insufficiency in such a patient would render surgery hazardous indeed

Fetal Zone of Adrenal Cortex: Significance. Within the first 3 days of life, the adrenal glands of a normal infant undergo dramatic histologic changes There is a loss of 50% of the adrenal mass and this is due to the rapid degeneration of the fetal zone of the adrenals The other elements of the cortex remain responsive to the pituitary stimulus (ACTH) as determined by a fall in circulating eosinophils and by increased urinary excretion of cortical hormones Normal premature infants show an increasing sensitivity and vigor in this adrenal response to stimuli as the days pass

Adults vs Infants. Adrenal Response To Surgery. It has been of interest to compare the plasma 17 21 hydroxycorticosteroid values obtained in infants with those in adults, all values having been reported from our own laboratory Free and conjugated plasma steroids in both infants and adults show a marked rise after surgery The infant response to trauma is not of the same magnitude as adults, but there is no doubt that adrenocortical activity is increased in these diminutive patients

The onset of response was rapid in both infants and adults and the decline from peak levels to control levels occurred during the 24 hours following surgery The major differences lay in the lower total values in the infants and in the much less marked increase in plasma hydrocortisone levels in these subjects

SUMMARY AND CONCLUSIONS

1 A microtechnique using a few cubic centimeters of plasma has been employed to determine concentrations of hydrocortisone in 11 infants

2 Increased adrenocortical activity after surgery in these infants has been demonstrated on the basis of increased plasma levels of the 17 21 hydroxycorticosteroids

3 The general pattern of response to surgical trauma was similar to the pattern of adults Although the magnitude is less, the qualitative and temporal response of adrenocortical activity is similar to adults

4 Adrenocortical replacement therapy does not appear to be indicated as a routine measure following surgery upon infants

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ENDOCRINE FACTORS IN THE ALTERED BLOOD COAGULATION POTENTIAL FOLLOWING SURGICAL STRESS*

JOHN A WILLIAMS AND RICHARD WARREN

Alterations in certain plasmatic factors involved in coagulation of blood appear to be among the most consistent of the homeostatic adaptations to surgical stress The importance of an augmented coagulation potential in guarding the integrity of the internal environment of the body was first emphasized by Cannon[1] who found in experimental animals that a variety of stimuli such as hemorrhage or infusion of epinephrine led to acceleration of the clotting time of whole blood

Previous studies of surgical patients in this laboratory[2] have shown that the efficiency of the prothrombin conversion reaction during clotting *in vitro* is regularly enhanced during the early postoperative period and that this alteration correlates well both in time and in magnitude with changes in peripheral blood levels of free 17 OH corticoids The present study was undertaken to evaluate further the validity of the hypothesis that 17 OH corticoids are an important component of the plasmatic thromboplastic mechanism

METHOD

To assess the *in vitro* effect of 17 OH corticoids on prothrombin consumption 40 ml blood samples were obtained by venipuncture in each of 11 normal subjects Each sample was divided equally between two clean glass test tubes containing either 0 1 ml of normal saline solution or 0 1 ml of a normal saline solution containing standard amounts of 17 OH corticoids (5 10 15 or 20 ug) and inverted once for mixing One milliliter samples were then immediately drawn from each tube and placed in siliconated test tubes for determinations of prothrombin consumption The

*From The Surgical Service West Roxbury Veterans Administration Hospital and the Department of Surgery Harvard Medical School Boston Aided in part by Public Health Service Grant No H 2036

remaining 19 ml in each tube were allowed to clot, and the concentration of 17 OH corticoid in the resulting serum was determined

Serum prothrombin times were determined by methods previously described[3] Concentrations of "free" 17 OH corticoids were measured by the method of Nelson and Samuels[4]

To evaluate the effect of 17 OH corticoids added *in vitro* on the clotting time of whole blood, 9 ml blood samples were drawn for each of 5 normal individuals and from each of 10 patients 30 minutes after intravenous injection of 40 mg of heparin Each sample was then divided equally among three groups of Lee White test tubes (1 ml in each tube), each group of three tubes containing either 0 1 ml of normal saline solution or 0 1 of normal saline solution containing standard amounts of 17 OH corticoids (5, 10, 15 and 20 ug) Similar studies were carried out using Lee White tubes containing 0 1 ml of normal saline solution, 5 ug of heparin, and standard amounts of 17 OH corticoid Clotting times in each case were measured by the Lee White method after a single inversion of the tubes to effect mixing

RESULTS

The data are presented graphically in Figure 1 and Figure 2

As Figure 1 illustrates, the addition of cortef to freshly shed blood resulted in increased prothrombin consumption (reflected by prolongations of the serum prothrombin times) The magnitude of the increase in prothrombin consumption appeared to be a function of the magnitude of the increase in serum 17 OH corticoid concentration produced by the admixture of this hormone Following the addition of 5 or 10 ug of cortef to 20 ml of blood *in vitro* the serum prothrombin times and serum 17 OH corticoid levels corresponded to the pattern which has been observed[2] in the early postoperative period

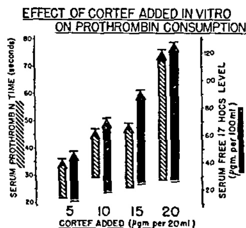


Fig 1 The effect of cortef added *in vitro* on prothrombin consumption The cross hatched arrows represent the changes in serum prothrombin times resulting from addition of standard amounts of cortef to the blood prior to clotting Solid black arrows indicate changes in serum 17 OH corticoid levels

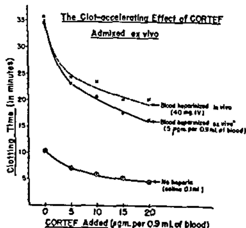


Fig 2 The clot accelerating effect of cortef admixed *ex vivo* Note that the thromboplastin like effect of cortef is modified but not abolished by heparin

The clot accelerating effect of cortef added to blood *in vitro* is shown in Figure 2 Again the effect appeared to vary systematically with variation in the amount of hormone added The presence of heparin *in the test* system, whether heparin had been added before or after withdrawal *of the* blood sample, modified but did not abolish this "thromboplastin like effect of cortef

DISCUSSION

The concept of a postoperative thrombophilic state is generally recognized Explicit definition of this concept in terms of postoperative alterations in the mechanisms of blood coagulation, however, has not been made It appears likely a number of physiologic factors are involved in conditioning the physiochemical state of the plasmatic protein moieties responsible for the prothrombin activity of the blood These observations suggest that adrenocorticoids may exert a "conditioning' influence on the prothrombin conversion reactions and may be the major physiologic determinants of this aspect of the total biologic response to stress It is suggested that postoperative thrombophilia is a manifestation of augmented adrenal cortical activity following surgery

SUMMARY

1 The effects of 17 OH corticoids added to blood prior to clotting *in vitro* on prothrombin consumption and on the clotting time with and without heparin were evaluated

2 It was observed that admixture of 'physiologic' amounts of 17 OH corticoid elevated serum concentration of 17 OH corticoids to levels corresponding to those observed in the early postoperative period and consistently resulted in acceleration of the clotting time and in increased prothrombin consumption

3 Adrenal cortical hormones may play an important role in conditioning this aspect of the total biologic response to stress

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SERUM PROPERDIN TITERS IN SURGICAL PATIENTS*

JERREL W BENSON, AND WILLIAM D HOLDEN

While speculation continues regarding the full significance of the Properdin System[1] as a factor in natural resistance to infection, properdin remains the most clearly defined antibacterial substance yet isolated from blood Experimental evidence based on differences in tolerance of animals to infection,[9] irradiation,[10] and shock,[3] relates abnormal susceptibility to serum properdin deficiency, and increased natural resistance to normal or rising properdin titers Appropriate treatment of such animals with properdin, under prescribed circumstances, enhances their tolerance to these conditions[4 5 10]

The common denominator of the various "substrates" which interact with properdin is polysaccharide in nature,[7] being found in bacterial filtrates, cell walls, and endotoxins, and also in mammalian tissues such as kidney, small intestine, pancreas, and lung[5 8] It is believed that the reactions of the properdin system with these substances indicate the mechanism by which it destroys bacteria, inactivates viruses, kills protozoa, and lyses abnormal erythrocytes Trauma, infection, or tissue anoxia may evoke the release of similar tissue polysaccharides which further deplete properdin in the host s blood[5 9]

Serum properdin levels below the normal range of 4 to 8 units per ml have been observed in patients with pneumonia, peritonitis, intestinal obstruction, hemorrhage, and various infections[6 7] Serum properdin titers remain normal after anesthesia, uncomplicated surgery, removal of the adrenal glands, or the spleen[7]

This report describes patients selected for study because the clinical conditions which they represent were frequently associated with low properdin levels in earlier pilot surveys From 3 to 20 properdin titers per patient were determined at intervals of one or two days by methods previously described[1] The usual number was 6 to 8 Data on patients grouped according to clinical categories are listed in Table I

Causes of peritonitis include perforations of peptic ulcer (12), gall bladder (2), and appendix (6) Among the patients having low properdin titers before surgery, 4 out of 6 died

All patients with obstruction of the small intestine had strangulated bowel which, with one exception, was resected Four of these patients had normal serum properdin titers prior to surgery which declined after 24 hours, returning to normal levels in most instances after 5 to 10 days The 2 deaths occurred in patients who developed very low titers (See Fig 1) Two of 3 patients with large bowel obstruction had low properdin titers No properdin was detected in the peritoneal fluid from 4 patients in this group

Three patients with thermal burns exhibited low properdin levels after wound infections developed Samples of blister fluid have been found to contain no more than one unit of properdin per ml

*From the Department of Surgery Western Reserve University School of Medicine and University Hospitals of Cleveland With the collaboration of Dr Louis Pillemer and the technical assistance of Leona Wurz and Jean Hower

Table 1 Serum Properdin Titers in Surgical Patients

CLINICAL CONDITIONS	TOTAL PATIENTS	PROPERDIN TITERS	MAJOR COMPLICATIONS WITH INFECTION	DEATHS
Peritonitis	20	Normal 8	1	0
		Low 12	12	6
Intestinal Obstruction	11	Normal 1	0	0
		Low 10	5	2
Thermal Burns		Normal 0	0	0
> 30% Body Surface	3	Low 3	2	0
> 2nd Degree				
Bullet Wounds		Normal 1	0	0
Abdomen & Chest	8	Low 7	3	1

Penetrating wounds of the thoracic or abdominal viscera were present in 7 of the 8 patients with bullet injuries Sites of injury were liver (2) colon and urinary bladder (1), pancreas and kidney (1), stomach and colon (1), liver, pancreas and spleen (1), lung, colon, kidney, and spleen (1) and shoulder (1) (followed by arteriovenous fistula of subclavian vessels) All developed subnormal properdin titers except the patient with the shoulder wound Six patients had titers below 1 unit per ml of serum 2 days after injury, although they received large volumes of blood, (See Fig 2) A young colored male survived similar but more extensive injuries than the patient depicted in Fig 2, although much injured tissue, (spleen, kidney, and transverse colon) was removed at surgery His properdin level was low for the first 3 postoperative days only, however, he developed infected wounds, empyema, and recurrent abdominal abscesses

DISCUSSION

Since serum properdin levels can be affected by both endogenous and exogenous polysaccharides the low levels associated with the conditions described could be due to interaction of properdin with either living or dead bacteria or with substances released by them, or with endogenous polysaccharides released from host tissues injured by trauma or disease Most patients who developed low serum properdin titers had infectious complications reflected by protracted fever, ileus, atelectasis, wound infections and delayed clinical recovery The lowest titers were seen from the second to the fifth postoperative days and their return to normal levels usually accompanied clinical recovery Low titers before surgery usually persisted after operation and were accompanied by the more serious complications and greater mortality rate The major complications referred to include wound disruptions, peritonitis empyema, pneumonia, septicemia and enterocolitis

A number of patients who were operated upon for gastrointestinal perforations or strangulation of the bowel showed precipitous declines in serum properdin titer after operation Marked transient depressions of serum properdin titer have also been noted in 6 instances coincident with incision and drainage of abscesses in or about the peritoneal cavity These observa

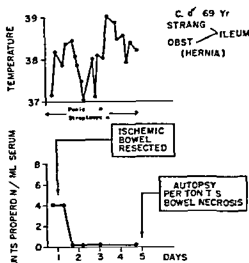


Fig 1 Properdin levels after bowel obstruction and resection

tions suggest that products of tissue necrosis and bacterial growth or death, which have been excluded from the blood stream by the inflammatory process or mechanical factors, may be released and combine with properdin following operative manipulation of diseased tissues

Stored citrated blood contains properdin in normal concentrations as judged by changes in serum titer of recipients The properdin content of fresh serum stored under usual blood bank conditions undergoes no appreciable loss after 45 days Fine[3] has observed marked depressions of the serum properdin titer in dogs made hypotensive by hemorrhage The failure of massive transfusions to elevate subnormal properdin titers in some patients and to prevent subsequent depressions of the titer in other patients when infections appeared after trauma, have been noteworthy in our studies More than 4000 ml of blood administered during surgery

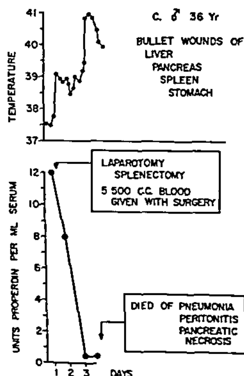


Fig 2 Properdin levels after visceral trauma and blood transfusions.

failed in 4 instances to elevate low properdin levels in patients with tic shock secondary to intestinal strangulation or suppurative peritonitis rger amounts of blood have been given to patients with hemorrhagic ck while their properdin titers remained normal but titers of such ients have usually dropped sharply within 48 hours It is concluded m such observations that the administration of whole blood is not an ctive means of restoring properdin in the presence of shock or over elming sepsis

SUMMARY

The rather frequent incidence of subnormal properdin titers is reported small groups of patients with peritonitis intestinal obstruction severe rmal burns and bullet wounds involving the thoracic and abdominal cera Mechanisms which may be involved in alterations of the serum operdin concentration under these circumstances are discussed

A causative relationship between operative manipulation of infected or ured tissues and declining properdin titers is suggested by observations patients with peritonitis and abscesses

Changes in serum properdin titers observed in patients with septic and morrhagic shock following massive transfusions of whole blood indicate t this is not an adequate means of restoring properdin in these nditions

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EFFECT OF SKIN PIGMENTATION ON FLASH BURNS IN HUMAN VOLUNTEERS*

JAMES W BROOKS, FREDERICK H SCHMIDT, RAY C WILLIAMS AND WILLIAM T HAM, JR

In a recent report from this laboratory the findings following flash burns on human volunteers of the white race were discussed [1] Our present report deals with a similar study carried out on human volunteers of the Negro race in order that the influence of skin pigmentation could be studied under controlled conditions This study has been directed toward the solution of the following broad questions

I What is the incident thermal dose (calories per unit area) required to produce first, second and third degree flash burns on unprotected human skin in the Negro race?

II What is the influence of the speed of delivery of heat on the severity of the burn lesion for a given thermal dose in the Negro race?

III What is the influence of the spectral quality on thermal injury in the Negro race?

Volunteers for this study were obtained from the Virginia Union University, Richmond Virginia and included 15 male and 7 female Negro students Preparing the arms for burning and details of the burning procedure have been described previously [1]

Thermal Energy Source The flash burn in all experiments was inflicted by a 24 inch Army searchlight utilizing a high intensity carbon arc, the radiation being focused on the burn aperture by an ellipsoidal mirror [2] A uniform burn 1 27 sq cm in area (7/16 inch diameter) was produced by techniques described previously, the incident thermal dose and thermal intensity were accurately determined and controlled Two types of exposure shutters provided thermal doses which could be delivered at a constant intensity (square wave) or with intensities varying rapidly with time (thermal pulse) Accurate timing of each exposure was electronically controlled Wave lengths below 3 200 A were excluded by a filter in an attempt to simulate atmospheric attenuation of the short wave lengths

Group 1. *Incident Dose Required to Produce First, Second, and Third Degree Burns in the Negro Race* Thermal doses of 1 8 2 4, 2 9, 3 3, and 3 7 cal per sq cm were delivered in approximately 540 milliseconds to the arms of 7 volunteers by means of a pulse of radiant energy delivered by a special shutter The thermal intensity attained its maximum value in approximately 180 milliseconds receding more gradually to zero level Spectral composition corresponded roughly to the wave lengths emitted by black body at about 5800° K Wave lengths below 3200 A were excluded by transmission of the flash through Corning filter 0 53 The spectral quality and time of exposure were held constant while the thermal dose was varied Table 1 and Figure 1 summarize briefly our observations

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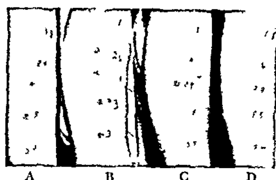


Fig 1 Photographs indicating progress of lesions on female subject of Group 1 Individual was classified as medium dark Numerical indications on arm indicate cal/cm² (A) 4 hours postburn (B) 3 days postburn (C) 9 days postburn (D) 14 days postburn

Group 2. *Influence of the Thermal Rate of Delivery on the Burn Lesion* The experiments in Group 2 were designed to investigate tissue response to a constant dose of thermal radiation when the intensity and time of exposure were varied A thermal dose of 3 7 cal/cm² was selected, and each of eight volunteers received 6 burns on the same arm Spectral quality again corresponded to that emitted by a black body at 5800°K, minus wave lengths below 3200 A The pulse shutter was used to provide exposure time of 0 5, 0 8, 1 1, 1 7, 2 8 and 3 4 seconds

In Table 2 the clinical estimation of degree of burn for the entire group is given for each exposure time

Figure 2 illustrates the progress of the burn lesions on a male volunteer in Group 2

Table 1 Clinical Assay of Degree of Burn Injury Produced by Various Thermal Doses under the Experimental Conditions Described for Group 1

DOSE FROM THERMAL PULSE IN CAL./Cm²	DEPTH OF BURN (CLINICALLY) ON UNPROTECTED NEGRO HUMAN SKIN
1 8	2°
2 4	2°
2 9	2°
3.3	Deep 2° or 3°
3 7	3°

Table 2 Effect of Thermal Rate of Delivery on Severity of Burn Lesion (3 7 cal/cm² by Thermal Pulse Shutter) in Negro Race

TIME (SECONDS)	DEGREE OF BURN (CLINICAL)
0.5	3°
0 8	3°
1 1	Deep 2°
1 7	Deep 2°
2 8	2°
3 4	2°

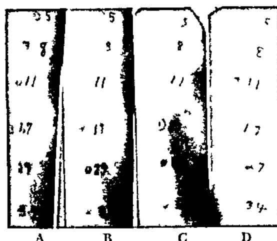


Fig 2 Photographs illustrating progress of lesions on male volunteer of Group 2 Figures on arm adjacent to lesions indicate time in seconds to deliver thermal dose of 3 7 cal/cm² by pulse wave technique (A) 4 hours postburn, (B) 3 days postburn (C) 9 days postburn (D) 14 days postburn

It is evident that the incident rate of input of radiant energy has an effect upon the severity of the burn lesion A dose of 3 7 cal/cm² delivered in 0 5 seconds produces a 3° burn in Negro volunteers, whereas the same dose delivered in 3 4 seconds produces only a 2° burn

Group 3. *Influence of Spectral Quality on Thermal Injury* The incident dose (3 3 cal/cm²) and the exposure time (0 54 sec) were maintained constant throughout the experiment while the spectral quality at each burn exposure was different This was accomplished by the interposition of appropriate Corning filters between the shutter mechanism and the burn aperture

The Corning filters used in this experiment are tabulated in Table 3 together with the clinical assessment of the degree of burn for each spectral composition The transmission characteristics of each filter are indicated in the table For example, filter 0 53 transmits better than 85% of all wave lengths beyond 3200 A, but exhibits a sharp cutoff just below this wave length All the filters listed transmit better than 85% of the radiation beyond their indicated cutoff

In the Negro race at least 2° burns were produced even when wave lengths beyond 6800 A were used There was a definite graduation in burn severity as the spectrum increased towards longer wave lengths but even the longest wave lengths studied produced 2° burns

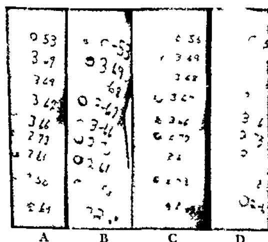


Fig 3 Photographs illustrating progress of lesions on a male, dark pigmented volunteer of Group 3 Figures on arm adjacent to lesions indicate Corning Filter numerical designation (A) 3 hours postburn (B) 3 days postburn (C) 9 days postburn (D) 14 days postburn

Table 3 Effect of Gradually Excluding White Light from the Radiation Spectrum of Carbon Arc with Resultant Greater Proportion of Infrared (3 3 Cal/Cm² in 0 54 Second by Square Wave Shutter)

FILTERS	DEGREE OF BURN
Corning Filter 0 53 3200 A and beyond	3°
Corning Filter 3 69 5350 A and beyond	3°
Corning Filter 3 68 5500 A and beyond	Deep 2°
Corning Filter 3 67 5650 A and beyond	Deep 2°
Corning Filter 3 66 5800 A and beyond	Deep 2°
Corning Filter 3 73 5900 A and beyond	2°
Corning Filter 2 61 6250 A and beyond	2°
Corning Filter 3 58 6500 A and beyond	2°
Corning Filter 2 64 6800 A and beyond	2°

Figure 3 is a series of photographs illustrating the typical findings in the volunteers of this group

We conclude that the spectral quality of radiant energy incident on exposed tissue of the Negro race has considerable influence on the severity of the burn lesion Burn trauma is a function therefore of spectral quality as well as of thermal dose and thermal rate of delivery Skin pigmentation plays an important role in determining the amount of incident radiation which is absorbed and seems to account in part for the dependence upon spectral quality

SUMMARY

Flash burns were produced on 22 Negro volunteers by means of a carbon arc operated at high current density A total of 146 individual flash burns were inflicted on the upper arms of the subjects, and these were studied with regard to thermal dose thermal rate of delivery, and spectral quality

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FACTORS AFFECTING THE DISTRIBUTION AND RETENTION OF RADIOACTIVE STRONTIUM*

D. P. GOEL, S. C. SKORYNA, L. YAFFE, AND D. R. WEBSTER

Radioactive strontium is one of the isotopes with long half-life produced during uranium fission. The long half-life of radioactive strontium is of particular importance because of the possibility of long term effects, after other isotopes with short half-life have decayed. The significance of radiostrontium in human pathology lies in its: (1) systemic effects, (2) deposition in high percentage in bony tissues, and (3) carcinogenic effects of relatively small doses. Studies of radioactive strontium are of special interest to surgeons because of the development of bone tumors observed in experimental animals following radiostrontium administration. In this experiment, the effects of parathyroid extract administration and low calcium diet on the retention and distribution of radiostrontium were studied with the ultimate objective of investigating the possibilities of decreasing the deposition of radioactive strontium in bone by means of hormonal factors.

METHOD

Male, adult, hooded rats, weighing 230 to 250 gm., of the Royal Victoria strain, were used in this experiment. Animals were kept in wire bottomed cages, 2 in each cage. The temperature of the room was maintained at about 78°F. The animals were divided into 4 groups which were treated as follows: *Group A.* Intraperitoneal injection of strontium-89 and normal Purina meal diet. *Group B.* Intraperitoneal injection of radiostrontium and low calcium diet. *Group C.* Intraperitoneal injection of radiostrontium, subcutaneous injections of parathyroid extract† and standard Purina meal diet. *Group D.* Intraperitoneal injection of radiostrontium, subcutaneous injections of parathyroid extract and low calcium diet.

Radiostrontium used in this experiment was supplied by the Atomic Energy of Canada as Strontium chloride ($SrCl_2$) solution. It contained varying proportions of Strontium-89 and strontium-90 mixture: the quantity of Strontium-90 did not exceed 10% of Strontium-89 present in the solution. The dose of radioactive strontium was 0.7 microcurie/gm. of body weight. The Strontium was 14 days old (17% decay) at the time of injection. Five subcutaneous injections of parathyroid extract were given to animals of Groups C and D. The first injection was administered immediately after radiostrontium injection. Three injections of 200 units were given on alternate days in the first week and 2 injections of 200 units in the second week.

Groups A and C were kept on normal Purina meal diet and water was supplied *ad libitum* According to information obtained from the manufacturer, Purina diet contains 1.8% calcium. Groups B and D were kept on low calcium diet starting 9 hours previous to administration of radiostrontium. Low calcium diet was specially prepared for this experiment and contained 0.2% of calcium.

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†Supplied by Dr Carl A Kuether, Lilly Research Laboratories, Indianapolis.

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Animals were sacrificed by a lethal dose of nembutal 15 days after the radiostrontium administration Liver, spleen, intestine, kidney, heart, lung skeletal muscle, skin epiphysis and diaphysis (from tibia) were removed and the amount of radioactivity measured by standard techniques using a Geiger Muller tube and associated electronic equipment All net counts per minute of the samples were compared with the measurements made on standards, prepared directly from the stock solution of radioactive strontium used for the injection

RESULTS

Fifteen days following the administration of radiostrontium (0 7 micro curie/gm body weight) 1 gm of epiphysis of 10 rats treated with 100 units of parathyroid extract and fed on normal Purina meal diet, (Group C) contained from 2 21 to 2 67% of injected dose, with a mean value of 2 43% *In those animals which received radiostrontium only, and were* kept on normal Purina diet, the activity per gram of epiphysis varied from 2 40 to 2 91% of the injected dose with a mean value of 2 67% Therefore, there was 8 98% more radiostrontium per gram of epiphysis in the animals, when compared to those which did not receive any parathyroid extract One gram of diaphysis of those animals which received parathyroid extract contained 20 8% less radiostrontium than the controls The mean percentage of the retained radiostrontium per gram of epiphysis of the animals in Group D (strontium 89 and Parathyroid extract and low calcium diet) was 3 70% of the injected dose, when compared to 4 21% in those animals which were kept on low calcium diet alone (Group B) a difference of 12 1% in these two groups In Group D there was 23 5% less retention of radiostrontium per gram of diaphysis than in the diaphysis of Group B animals which did not receive any parathyroid extract Small amounts of radiostrontium were detected in the liver, spleen, kidney, intestine, skin skeletal muscle, heart muscle and lung With a few exceptions there was practically no activity present in liver, spleen, heart skin skeletal muscle and lung of Groups A and B animals In these 2 groups there was some activity present in intestine and kidney which probably

Table 1 Distribution of Radiostrontium 15 Days After Intraperitoneal Administration in Rats Kept on Normal Purina Diet

	GROUP A STRONTIUM 89 PLUS NORMAL PURINA MEAL DIET									
TISSUE	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6	RAT 7	RAT 8	RAT 9	RAT 10
Liver	00062	00124		00594	00293		00113			00117
Spleen										00031
Intestine	00039	00123	00239		00083	00139	00079	00211	00151	00079
Kidney	0016	0043	0041	0022	0031	0041	0018	0048	0014	0031
Heart					00053					
Lung	00064	00139							.00059	
Skin										
Muscle										
Epiph	2 75	2.59	2 69	2 91	2 42	2 67	2 91	2.67	2 71	2 40
Diaph	0 682	0 691	0 722	0 754	0.564	0.562	0 756	0 726	0 687	0 682

(Values in percentage of injected dose per gram of wet weight of tissue)

Table 2 Distribution of Radio Strontium 15 Days After Intraperitoneal Administration in Rats Kept on Low Calcium Diet

	GROUP B STRONTIUM 89 PLUS LOW CALCIUM DIET									
TISSUE	RAT 11	RAT 12	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	RAT 18	RAT 19	RAT 20
Liver										
Spleen				00119						
Intestine		00113	00217	00339			.00117	00214	00319	00021
Kidney	0056	0036	0061	0036	0081	0031	0061	0061	0056	.0061
Heart										
Lung	00169	00119	00101							00117
Skin				00061	00039					00039
Muscle										
Epiph	3 69	4.26	5 72	3.57	3 83	5 64	3 65	3 60	4.35	3 83
Diaph	0 813	0 899	1 61	0 762	0 793	0 823	0.887	1.51	0 774	0 793

(Values in percentage of injected dose per gram of wet weight of tissue)

Table 3 Distribution of Radiostrontium 15 Days After Intraperitoneal Administration to Rats Kept on Normal Diet which Received Parathyroid Extract

	GROUP C STRONTIUM 89 PLUS PARATHYROID EXTRACT PLUS NORMAL PURINA MEAL DIET									
TISSUE	RAT 21	RAT 22	RAT 23	RAT 24	RAT 25	RAT 26	RAT 27	RAT 28	RAT 29	RAT 30
Liver	0023	0012	0039	0016	0024	0025	0010	0035	0020	0024
Spleen	0018	00061	0016	0043	0017	0018	00065	0012	00031	0029
Intestine	0027	0038	0062	0024	0045	0029	0036	0069	0024	0038
Kidney	036	0075	014	0071	074	036	0075	019	0071	069
Heart	0023	00089	00065	00032	0029	0023	00087	00067	00041	0020
Lung	0037	0015	0038	00062	0073	0034	0018	0039	00062	0072
Skin	0028	0029	00094	00062	0033	0021	0036	00094	00062	0033
Muscle	0084	0012	0012	0023	0037	0084	0014	0010	0039	0021
Epiph	2 67	2.24	2.57	2 49	2.21	2.21	2 67	2.34	2 47	2 49
Diaph	0 481	0.563	0 494	0 533	0.531	0 480	0.564	0 499	0.528	0.531

(Values in percentage of injected dose per gram of wet weight of tissue)

Table 4 Distribution of Radiostrontium 15 Days After Intraperitoneal Administration to Rats Kept on Low Calicum Diet and which Received Parathyroid Extract

	GROUP D STRONTIUM 89 PLUS PARATHYROID EXTRACT PLUS LOW CALCIUM DIET									
TISSUE	RAT 31	RAT 32	RAT 33	RAT 34	RAT 35	RAT 36	RAT 37	RAT 38	RAT 39	RAT 40
Liver	0091	0026	0023	0063	0018	0091	0024	0035	0059	0022
Spleen	0033	00061	0029	0024	0018	0031	00061	0031	0024	0018
Intestine	0045	0037	0043	0022	0022	0045	0039	0045	.0022	0022
Kidney	016	0041	096	028	083	015	.0041	097	029	082
Heart	00161	0011	0062		00062	0016	0012	0061		
Lung	0024	0023	0080	0012	00062	0024	0021	0082	0015	00062
Skin	0012	0031	0023			0015	0028	.0023		
Muscle	0031	0023	0018			0031	0025		0016	
Epiph	4.58	3 45	3.98	3.31	3 19	3 18	3 48	3.28	4.59	3.98
Diaph	0 661	0 782	0 694	0 666	0.926	0.661	0.826	0 765	0.693	0 784

(Values in percentage dose per gram of wet weight tissue)

represents excretion values The activity in soft tissues in Groups C and D animals which received parathyroid extract although negligible when compared to the skeletal tissue was higher than in Groups A and B

DISCUSSION

Tweedy[1] studied the effects of parathyroid extract on the metabolism of radiostrontium and found that 2 injections of 500 units of parathyroid extract 24 and 1 hour before the administration of radiostrontium resulted in decreased retention of radiostrontium in the femurs and increased excretion and retention in the kidneys In our experiment the parathyroid extract was *given following the administration of radioactive strontium* with the object of influencing the excretion of the isotope The results shown in Table I indicate that injection of parathyroid extract in 5 divided doses starting immediately after the administration of radioactive strontium *resulted in decreased retention of the isotope both in epiphyses and dia*physes when compared to the control group There was 8 98% lower retention in per gram of epiphysis and 23 5% in per gram of diaphysis in Group C animals (radiostrontium plus normal diet) Higher values of *radioactivity in the intestine and kidney in animals receiving the para*thyroid extract indicate that the excretion of radioactive strontium in these animals was increased

Rats which were kept on low calcium diet (Groups B and D) showed higher radioactivity per gram of epiphysis and diaphysis when compared to the animals receiving normal diet (Groups A and C) These results correspond to the findings of Kidman *et al*[2] in rabbits and Copp *et al*[3] in rats One would expect that animals receiving parathyroid extract alone (Group C) would excrete more radioactive strontium when compared to those which were not receiving it (Group A) This was the case in our experiment However when low calcium diet was given to the animals receiving parathyroid extract (Group D) actually a 35 1% increase in radioactivity per gram of epiphysis was observed This result might be explained by the fact that in this group the original retention of radioactive strontium in bone was higher due to low calcium diet

The high concentration of radioactive strontium in the intestine and kidney observed in Groups C and D might be explained by increased excretion of the isotope under the influence of parathyroid extract The increased concentration of isotope in liver spleen heart skeletal muscle lung and skin in these groups appears to be due to secondary deposition of the isotope from circulation produced by parathyroid extract In Groups A and B soft tissues except the intestine and kidney did not show any radioactivity in the majority of the animals 15 days after the administration of the isotope

CONCLUSIONS

The administration of parathyroid extract and low calcium diet described in this experiment represents an attempt to modify the uptake and retention of radioactive strontium by hormonal factors The low calcium diet alone increased the uptake of the isotope by the bone tissue The parathyroid extract decreased the uptake by epiphyses and diaphyses When animals kept on low calcium diet were treated with parathyroid extract the uptake

of radioactive strontium was increased when compared to the animals receiving parathyroid extract and normal diet It might be speculated that although parathyroid extract increased the excretion of the isotope, the higher uptake by bone tissue in these animals was due to the increased extraction of radio strontium from the blood instead of calcium which was present in lesser amounts due to low calicum diet Further studies on the effects of hormones on radioactive strontium metabolism are necessary to investigate the possibilities of decreasing the deposition of the isotope in the bony tissue

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MAST CELL ACTIVITY UNDER VARIOUS FORMS OF STRESS*

J LEONEL VILLAVICENCIO AND GEZA DE TAKATS

This study was undertaken for the purpose of (1) studying the normal basic behavior of the mast cell (2) observing the histologic effects of preloading the mast cells with heparin (3) watching the effect of ACTH on the normal and on the previously heparinized mast cell and (4) evaluating other agents which might possibly degranulate mast cells and release their content Obviously the staining method had to be carefully standardized since the vulnerability of mast cells to minimal stimuli including hypotonicity and change in pH are sufficiently known Evidently the mast granule is an intracellular osmometer from which heparin can be released as hydration occurs [1]

Opinions as to the effect of ACTH acting through cortisone on the mast cells is divided Asboe Hansen Stuart Cavellero and Braccini Bloom Fulton and Maynard believe that this hormone decreases the number of and degranulates the mast cells [2 3 4 5 6] Negative findings were reported however by Schoch and Glick [7] and by Devitt Pirozynski and Samuels [8] Hardly two investigators have used the same staining methods and the conflicting results may be due at least in part to technique of fixation and staining

METHOD

The recently published simple and rapid staining procedure of Smith and Atkinson [9] was used Normal albino rats weighing from 175 to 200 grams were anesthetized with intraperitoneal nembutal After a laparatomy a glass slide was slipped into the abdominal cavity then a freely

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*From the Department of Surgery University of Illinois College of Medicine Supported by the Chicago Heart Association with the assistance of Dr Hubert Catchpole of the Department of Pathology on staining methods

movable intestinal loop was gently picked up and the mesentery spread on the slide with a minimum of trauma. With a clean, quick cut of sharp scissors, this segment was freed of its mesenteric attachment, fixed in absolute alcohol for 8 hours and stained by immersing the slides for 15 seconds in 0.5% solution of toluidin blue. The slides were then gently rinsed for 1 to 2 seconds with 95% alcohol, cleared through xylene for 12 hours and mounted with clarite under a glass overslip. Care was taken to strictly observe these time relationships and to never vary the concentration of the basic dye.

The mast cells of the rat mesentery were then observed under the following conditions:

(1) In a group of 10 normal rats, 20 fields were counted of each mesentery, care being taken to count 10 fields in the perivascular areas and 10 fields in avascular connective tissue. Since mast cells are encountered in various stages of development, maturity, and degranulation, the percentage of "abnormal" mast cells was noted. All cells showing karyorrhexis, karyolysis, vacuolization and rupture of cellular membrane with a halo or scattering of granules were counted as abnormal. An N.A.66 Spencer objective X93 and an X15 ocular lens was employed. This magnification was used in all the other counts.

(2) In a group of ten rats, 10 mg. of ACTH was injected intramuscularly and the mast cells observed 4 hours after the administration of the drug.

(3) A third group of 10 rats was given 4 mg. of heparin intramuscularly for 5 days. On the fifth day, 10 mg. of intramuscular ACTH injection was given 4 hours after the last dose of heparin. Mast cells were observed 4 hours after the injection of ACTH.

(5) 20 animals in this group were given nitrogen mustard. They were divided in 4 groups, each containing 5 rats. Each subgroup contained three treated animals and two controls. Nitrogen mustard was given intravenously in doses of 0.5 mg. per kilogram body weight.

The controls received isotonic solution of sodium chloride of equal volume, i.e. 1 cc. The injections were given and the mast cells were studied 20 minutes later according to the following pattern:

a) *The first day* all animals received nitrogen mustard or saline. The first group of 5 animals were explored and their mesenteries studied for mast cells.

b) *The second day* all remaining animals received a second dose of nitrogen mustard and the controls received saline solution. A second group of 5 animals were anesthetized and their mast cells observed.

c) *The third day*, a third dose of the drug was given to all the remaining rats, the controls receiving saline solution. Another group of 5 rats mesenteries were studied.

d) *The fourth day*, the last group of rats was injected with nitrogen mustard or saline respectively and their mesenteries were observed.

Thus, we had rats receiving 1, 2, 3, and 4 injections of nitrogen mustard on successive days with appropriate control injections of saline.

(6) In a sixth group of ten rats, a non anaphylactogenic polysaccharide piromen (Baxter) was injected in a single dose of 0.5 micrograms. Mast cell counts were done at the height of the rectal temperature which proved to be at three hours.

(7) A last group of ten rats was given 700 Roentgen units as a total body irradiation They were observed 25 days after the administration of this dose

RESULTS

(1) In the group of normal controls, circular fusiform or oval cells were observed measuring from 5 to 30 micra in diameter Their cytoplasm was homogenously packed with metachromatic granules Most of the cells exhibited a central nucleus The cells were distributed mainly around blood vessels, often in rows parallel to them This perivascular arrange ment was more frequent than their distribution in avascular areas The perivascular mast cells showed orthochromatic staining with some meta chromatic granules, whereas the ones in the connective tissue showed over whelmingly metachromasia (the purple red color) an observation stressed previously by Riley and West[10] The total number of cells counted was *36,745*, *23%* of which were vacuolized, degranulated or disrupted

(2) In the group of rats receiving ACTH, *30,020* cells were counted Vacuolization, degranulation and disruption were observed in *52 8%* of the cells confirming the findings of Asboe Hansen[2] and others

(3) After five days of heparin administration, the mast cells stained much more deeply than in the normal controls, a fact more readily seen in kodachrome slides There also seemed to be a numerical increase in metachromatic cells readily demonstrable under low power In this group, a total of *66,423* cells were counted with a percentage of disruption amount ing to *31 8%*

(4) In the group heparinized and then injected with ACTH, both the absolute number was increased from the normal (59,427 cells) and the amount of abnormal cells was higher *66 9%* There seemed to be, however, a slight decrease of total cells when compared to those given heparin alone, roughly 7,000 cells in 20 fields A great number of metachromatic granules were scattered in the connective tissue, the granules often visible at great distance from the parent cell and vacuolization was prominent Around some cells a purplish halo was visible consisting of nongranular material just like the phenomenon observed by Paff and Bloom *in vitro*[11] indicating that metachromatic material has left the cell in solution

(5) In the group given nitrogen mustard, the highest amount of mast cell disruption was observed In the 4 groups receiving this drug on successive days, the percentage of cellular abnormality was 90 6%, 86 1%, 89% and 87% respectively The absolute number of cells decreased from a control of around 5,000 cells to 3 527 3 245, 2 894, and 2,467 cells respectively While the first injection of NH_2 produced the highest dis ruption of the mast cells (90 6%), the greatest structural damage appeared in the last group which had received four doses of nitrogen mustard

(6) After the intravenous injection of piromen, the rats' rectal tem peratures rose from 97 5° 98 5°F to a peak of 102°, 103°, and 103 5°F at 3 hours At this time, the mast cells were counted and observed The total number counted in 20 fields was 32 597, and the percentage of dis ruption was 57 6% Mast cell disruption after piromen has also been seen by Dougherty[12] and Stuart[3] The majority of abnormal cells had burst

Table 1 Total Count and Percentage of Disrupted Mast Cells Under Various Experimental Conditions

EXPERIMENT	TOTAL CELL COUNT*	PERCENTAGE OF ABNORMALS
Control	36 745	23
Heparin	66 423	31 8
ACTH	30 020	5[illegible] 8
Radiation	*32 407*	*54 6*
Piromen	32,597	57 6
Heparin and ACTH	59 427	66 9
Nitrogen mustard	11 313 (9428)	88

*20 fields counted for each animal 200 fields for a group of ten In the group injected with nitrogen mustard slides of 12 animals were counted in a total of 240 fields This was corrected to 10 animals in the brackets

and their metachromatic content was scattered through the perivascular and avascular areas Vacuolization was prominent

(7) 25 days after total body irradiation with Roentgen ray the mast cells showed degeneration with development of vacuoles extrusion of granules and poor metachromatic staining *32,407* cells were counted and these changes were observed in *54 6%* of the counted cells It should be noted that according to Smith and Lewis by this time the cells are beginning to recover

Table 1 *summarizes the numerical data*

DISCUSSION

The limitations of a histologic approach to the functional activity of a set of cells or an organ are obvious Yet we feel that such studies may give an insight into the behavior of mast cells So little is known about the significance of these cells around malignant tumors[13, 14] or in nutritional deficiencies[15] that a multifaceted approach including histologic isotope and *in vitro* culture studies are rewarding

SUMMARY

With the help of a simple easily standardized staining method the behavior of mast cells was studied on the rat s mesentery In normal controls it was possible to observe maturity, aging and death of cells though obviously the act of fixation and staining may cause artefacts ACTH radiation piromen and nitrogen mustard caused much more than the normal amount of vacuolization and disruption of the mast cells Heparin definitely deepened the staining quality of these cells and in*creased their number* ACTH *readily* emptied the granules of the heparinized mast cells

These findings indicate that mast cell *activity can* be activated or increased by external stimuli Loading of mast cells with heparin may make them available in times of stress Thus a type of prophylactic preoperative anticoagulant therapy may find its justification[16]

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INTRA AND EXTRACELLULAR SHIFTS OF WATER AND ELECTROLYTES DURING THE ACUTE RADIATION SYNDROME*

M DON TURNER ROBERT D SLOAN JAMES C GRIFFIN JR AND TOM D NORMAN

Heavy exposure of the abdominal area of animals to ionizing radiation results in rapid death The survival time of mice following supralethal irradiation is three to five days[1] The survival time in larger mammals is somewhat more than in mice and in humans it appears to be much longer The rapid death following irradiation of the abdominal area is known to be due to intestinal damage[2] and for this reason the acute radiation

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syndrome has come to be known as the *intestinal* radiation syndrome The acute radiation syndrome can be produced by irradiating heavily any large segment of the bowel [1] Apparently infection plays no major role in contributing to death following bowel irradiation,[2] and it has not been established that fluid and electrolyte disturbances are the major cause of death The following studies represent a preliminary investigation of fluid and electrolyte alterations following specific bowel irradiation

METHOD

Method of Irradiation: Approximately 60 inches of the small bowel of 5 dogs were exposed beginning 6 inches from the ileocecal valve and extending proximally This bowel segment represented about 50 to 60% of the entire small bowel of the dogs The intestinal segment was brought through a rectagular opening in a lead shield (7 mm thick) which completely covered the bodies of the animals A small lead shield was placed over the opening in the lower shield, leaving only the intestinal segment exposed A dose of 4000 r was delivered to the gut segment by an X ray unit operating at 110 KV, 7 ma No filter was used because of the particular geometry of the exposure

Fluid and electrolyte studies were made on each animal before and again 7 days following irradiation Total body water was determined as the distribution volume of N acetyl, 4 aminoantipyrine according to the method of Brodie, *et al* [3] The inulin space and the concentrations of sodium, chloride, potassium and water in skeletal muscle, plasma, and red cells were determined simultaneously with the total body water Electrolyte concentrations in the inulin space were corrected since extracellular fluid is an ultrafiltrate of plasma These measurements enable estimation of fluid and electrolyte changes in the inulin space, fluid alterations in the intracellular water, and shifts of electrolyte to and from cells of skeletal muscle The calculations involved the following assumptions (1) N acetyl, 4 aminoantipyrine is distributed equally in the extra and intracellular compartments, (2) inulin is limited to the extracellular compartment, (3) the inulin space of dog skeletal muscle is approximately 10 per cent of the skeletal muscle mass [4] All animals received 600,000 units of penicillin and 0 5 gram of streptomycin daily after exposure in order to eliminate infection as nearly as possible

RESULTS

All animals exhibited a moderate to severe diarrhea from the third to tenth day following irradiation with a peak on about the fifth day Water loss from the inulin and NAAP spaces after radiation is shown in Table 1 The total water loss approximates body weight loss in all except one animal (Dog 4)

The decrease in intracellular water was relatively greater than the fall in body weight, indicating a state of intracellular dehydration The mean total body water in the control animals was 60 2% of the body weight as compared to a mean of 47 1% after radiation The intracellular fluid volume averaged 42 6% of body weight in the control studies but only 29 2% after bowel irradiation These values indicate a severe cellular dehydration The inulin space, before and after exposure, was altered in

Table 1 Water Loss from the Inulin and NAAP Spaces After Bowel Irradiation

DOG NO	NAAP SPACE (% WT)	NAAP SPACE (LITERS)	INULIN SPACE (% WT)	INULIN SPACE (LITERS)	INTRACELLULAR SPACE (% WT)	INTRACELLULAR SPACE (LITERS)	LOSS FROM NAAP SPACE (LITERS)	LOSS OF BODY WT (KG)	LOSS FROM INULIN SPACE (LITERS)	LOSS FROM INTRACELLULAR SPACE (LITERS)
1 *C	55 9	10 16	19 7	3 26	38 0	6 90	3 03	4 10	0 73	2.30
R	50 5	7 13	17 2	2 53	32 7	4 60				
2 C	61 4	11 13	16 3	3 05	45 0	8 38	5 90	5 90	0 76	5 14
R	43 5	5 53	18 0	2 29	25 5	3 24				
3 C	60 1	9 83	18 2	2 92	42 3	6 91	4 40	4 29	0 53	3 87
R	45 0	5 43	19 0	2 39	25 2	3 04				
4 C	61 5	12 88	17 6	3 45	47 2	9 43	5 95	3 63	0 41	5.55
R	42 4	6 93	18 6	3 04	23 8	3 88				
5 C	59 1	10 73	16 9	3 02	40 7	7 39	2 84	3 63	0 77	1 75
R	51 3	7 89	15 5	2 25	38 8	5 64				

*C control R seven days after irradiation

proportion to body weight changes, with a mean of 17 7% of body weight before and 17 6% after irradiation

The loss of sodium, chloride, and potassium from the inulin space is shown in Table 2 These electrolyte losses are in proportion to the amount of water lost from the inulin space since the concentrations of these ions remained unaltered after intestinal irradiation Since external balance studies were not done it is not possible to account for the total electrolyte loss

Table 2 Losses of Sodium, Potassium, and Chloride from the Inulin Space After Bowel Irradiation

DOG NO	SODIUM (mEq)	POTASSIUM (mEq)	CHLORIDE (mEq)
1	95 7	1 8	73 1
2	99 6	3 3	85 0
3	88 6	2 5	78 9
4	53 9	0 7	51.5
5	131 1	0 68	86.2

Table 3 shows the apparent shift of electrolyte into skeletal muscle cells These values were calculated from ion concentrations of extracellular water, ion concentrations of extracellular water, ion concentrations of skeletal muscle, muscle water concentrations, and from the previously mentioned assumption that the inulin space of dog skeletal muscle is approximately 10% of the wet muscle weight

Table 3 Change in Skeletal Muscle Na, K, and Cl After Bowel Irradiation

DOG	SODIUM		POTASSIUM		CHLORIDE	
NO	(mEq /kg)	(% INCREASE)	(mEq /kg)	(% INCREASE)	(mEq /kg)	(% INCREASE)
1	+ 7 6	33 1	+3 0	3 0	+5 9	35.5
2	+20 0	71.3			+7 0	37 1
3	+ 7 9	48 1	+0 18	0.3	+3 04	23 7
4	+12 9	86 0	+7.2	7.3	+1 6	12 6
5	+ 9.2	55 4	+7 6	8.3	+0.2	1 6

The relatively large increase of sodium and chloride in the intracellular space was expected to occur as in most cases of prolonged diarrhea The expected fall in intracellular potassium was not apparent from these data Actually, the intracellular potassium concentration increased slightly A decrease in cellular potassium content probably did occur, if the cellular potassium content had remained the same, then a marked increase in potassium concentration would be seen due to the large water loss from the intracellular compartment

The rise in tissue electrolyte concentrations was probably partly the

result of pure water loss from the intracellular compartment and partly the result of actual electrolyte shifts No significant alterations in red cell electrolytes were observed although electrolyte concentrations were slightly above control values

DISCUSSION

Irradiation of a segment of small bowel was done in an attempt to obtain a picture of the results of pure bowel irradiation Serial blood and bone marrow hematology were studied in similarly irradiated animals along with the studies of post irradiation bowel pathology This work will be reported elsewhere

Animals irradiated as described here expire from the fifth to the tenth day after exposure This critical period correlates well with the period of diarrhea After about the tenth day the diarrhea diminishes and the stools become almost normal though still loose If the animal survives as long as 10 days it will live for several months Several dogs have been observed as long as 3 months and then sacrificed in order to study the gut pathology Long term survivors always developed typical irradiation ulcers in the bowel segment exposed to x ray These ulcers would probably finally perforate and cause death of the animal

SUMMARY

Marked alterations in body fluid and electrolyte content were observed following specific irradiation of the distal 50 to 60% portion of the small bowel Irradiation of the entire bowel would certainly be expected to result in much greater changes The loss of body water clearly demonstrates a marked dehydration with the bulk of the water lost from the intra cellular compartment Fairly large amounts of sodium and chloride were lost from the extracellular fluid and probably large amounts of sodium potassium and chloride accompanied the intracellular water loss How ever it is difficult to evaluate these findings until a more complete study of the acid base picture is done On the basis of these studies we are of the opinion that fluid loss is a major cause of death in animals subjected to heavy bowel irradiation

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THE SURVIVAL OF TRANSFUSED MARROW IN THE X-IRRADIATED RABBIT AS INDICATED BY SEX-DIFFERENTIATED LEUCOCYTES*

K. A. Porter and J. E. Murray

The ability of intravenous bone marrow to modify the effect of whole body X-irradiation in mice, rats, hamsters and guinea pigs has been demonstrated by numerous workers. Furthermore, it has been shown that this modification is dependent upon survival of the introduced marrow cells and repopulation by them of the host's depleted hemopoietic tissues.[1]

Following Barr's[2, 3] demonstration that a nuclear sex difference was present in almost all tissues of many mammalian species including man, a comparable morphological sex difference was shown in polymorphonuclear leucocytes in stained blood films from humans,[4] rabbits[5] and dogs.[6]

In this report marrow transplantation experiments with rabbits are described in which this sex difference in the leucocytes has been used to identify the homologous cells and their descendants after injection. By injecting marrow from a female animal intravenously into an irradiated male, survival and proliferation of the donor cells in the host was deduced if typical female cells could later be identified in the host's peripheral blood and marrow.

As previous work[7] had shown that in the rabbit after an X-ray dosage which permitted a large percentage of successful homotransplants (900–1,000r) a high mortality occurred from initial shock and later gastric ulceration and perforation, divided doses of irradiation and post-irradiation antibiotic therapy were used to minimize these complications.

METHOD

Young adult male New Zealand white rabbits weighing 2 to 3 kg. were used as recipient animals.

X-rays were produced by a 250 kVp Phillips machine operating at 15 mA; a 0.4 mm. Sn, 0.25 mm. Cu and 1.0 mm. Al filter was used giving a half-value layer in copper of the filtered beam of 2.5 mm. The exposure rate was 50r per minute at 50 cm. center of animal to target distance, measurement being made in air with a Victoreen ionization chamber. The marrow for post-irradiation injection was obtained from homologous young adult female rabbits. After the donors had been killed by intravenous nembutal the marrow from both femora, tibiae and humeri was removed and suspended gently in cold saline. This suspension was then centrifuged at 600 r.p.m. for 5 minutes and the supernatent fat discarded. The volume was adjusted so that 10 ml. contained 1,200 (x 10[6]) nucleated cells. Marrow injections were given within 1 hour of the death of the donor and were administered intravenously into the marginal ear vein of the recipient

*From the Laboratories for Surgical Research, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts. Supported by a contract (AT (30-1)-735) between the Atomic Energy Commission and the Peter Bent Brigham Hospital and by grants (H-2791 and H-1771) from the United States Public Health Service. With the technical assistance of Mrs. G. Shohet and Mr. J. Rahilly.

rabbit 1 to 3 hours following irradiation Controls each received 10 ml of saline intravenously

Total leucocyte counts and differentials, hemoglobins and micro-hematocrits were determined on the irradiated animals every 2 days for the first 30 days and once per week thereafter Blood films were taken from the ear at the same time intervals, stained by Wright's method and the polymorphonuclear leucocytes examined Female leucocytes are characterized by a nuclear appendage called by Davidson and Smith[4] a 'drumstick", consisting of a well defined chromatin nodule about 1 5 u in diameter, joined by a single fine chromatin strand to one lobe of the nucleus These are present in approximately 1 in 14 of the polymorphonuclear leucocytes in each blood film in female rabbits[7] Before accepting that the leucocytes were at least in part derived from the transplanted female bone marrow it was arbitrarily decided that a minimum of 6 cells showing typical "drumsticks" should be found

All animals dying were autopsied, and marrow from the mid point of the shaft of the right femur and the thymus, spleen, mesenteric lymph node and appendix were taken and fixed in Helly's fluid

All animals were exposed to 1,100r whole body X irradiation They were then divided into 3 groups *Group 1* consisted of 40 rabbits which were given marrow intravenously and, starting on the day of the last irradiation, they were also given 70 to 75 mg tetracycline hydrochloride (Lederle) per day *Group 2* The 20 rabbits in this group were given tetracycline following irradiation, but no bone marrow *Group 3* consisted of 20 animals which received neither antibiotic nor bone marrow after irradiation

RESULTS

Group 1. 65% of the animals showed a successful marrow transplant as indicated by rapid restoration of the leucocyte count to normal and the appearance of female cells in the peripheral blood, 15 4% of these subsequently died as the result of gastric perforation, 19 3% died after rejection of the marrow transplant by the host, 27% died at 33 to 40 days following progressive wasting diarrhea and a leucocytosis, with marked lymphoid atrophy but with an intact and functioning marrow transplant and 38 2% remained alive and well for 7 months with the marrow transplant intact and still contributing female cells to the peripheral blood Forty per cent of all the animals in Group 1 survived the 7 month experimental period but only 63% of these still had persistent marrow transplants at this time, the other 37% having regenerated their own bone marrow

Group 2. Twenty five per cent of these rabbits regenerated their own bone marrow and survived

Group 3. Ten per cent of these animals were alive and well at 7 months having regenerated their own bone marrow

DISCUSSION

The data obtained in these experiments add to the considerable evidence recently accumulated[1] that in irradiated animals treated with homologous†

†Homologous from a different strain of the same species as distinguished from isologous from the same inbred strain and heterologous from a different species

or heterologous marrow, the introduced cells survive and repopulate the host's depleted hemopoietic tissues

Also further light is shed on the late mortality which occurs in irradiated animals treated with homologous or heterologous marrow It is evident that under the conditions of this study, although some delayed deaths were undoubtedly due to rejection of the transplant by the host s regenerated antibody producing system, many deaths were due to some other cause

In this latter group (the 27% dying at 33 to 40 days) the syndrome of wasting and diarrhea appears similar to that seen in mice by Congdon and Urso[9] Our results exclude destruction of the marrow transplant by a delayed immunological response of the host to the foreign cells as the mechanism in this group of late deaths The hypothesis that the graft is forming antibodies against the host[10] certainly cannot be excluded on the present evidence and remains a possibility, as also does the supposition that these deaths are the result of delayed intestinal damage from irradiation[11]

The great advantage of the nuclear 'maker technique used in this work is its simplicity Other labelling methods for use in homologous (as distinct from heterologous) marrow transplantation studies involve either a supply of animals of specific antigenic type[8] or of animals with some distinctive chromosome pattern as in the T6 strain of mice used by Ford *et al*[1]

As it is probable that a similar morphological sex difference will be shown to be present in the neutrophils of many mammalian species other than the three mentioned earlier, the method described may prove useful in similar work with other animals and in any clinical studies that may be evenutally attempted

SUMMARY

1 Use of the sex difference in morphology of polymorphonuclear leucocytes to indicate survival of marrow homotransplants is shown to be a simple reliable 'marker" technique

2 In 65% of irradiated male rabbits given injections of homologous female bone marrow, a successful marrow transplant occurred as shown by the appearance of female leucocytes in the peripheral blood, but by 7 months over half these animals had died

3 Analysis of the late mortality which occurred in these animals with successful transplants showed that although some deaths were undoubtedly due to rejection of the transplant by the host's regenerated immune system, this mechanism could be excluded in the large group dying after suffering from wasting and diarrhea Reaction of the homograft against the host, or delayed effects of radiation damage remain as possible explanations

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Cancer

CANCER CELLS IN THE CIRCULATING BLOOD*

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RUTH G. MCGRATH, STELLA NANOS, AND WARREN H. COLE

Reports of the systematic search for cancer cells in the circulating blood have been few[1-3] due to the technical difficulty in the separation and cytologic identification of relatively few cancer cells from the numerous formed elements of blood. This study is a continuation of work begun four years ago[4] and concerns the isolation of cancer cells from the blood stream and the role of surgical manipulation upon the occurrence of these cells in the circulating blood.

METHOD

Collection of Blood Samples. *Each 10 cc. sample of heparinized blood* was processed immediately. Samples were obtained from one or all of three venous sources: (1) peripheral blood samples from antecubital vein venipuncture, (2) direct needle aspiration of blood from a vein draining the tumor site, (3) aspiration of blood from a cardiac catheter inserted into the major venous trunk draining the tumor area.

Isolation of Cancer Cells. The isolation of cancer cells from the formed elements of blood is accomplished by taking advantage of small differences in their specific gravities. The erythrocytes are removed by accelerated sedimentation using fibrinogen.[5] The supernatent plasma containing the cancer cells and leukocytes is then decanted and layered over isosmotic bovine albumin solution adjusted to a specific gravity of 1.065.[6] Following *centrifugation the majority of the tumor cells, which are lighter than* the adjusted albumin, are collected at the plasma-albumin interface. The tumor cells are then aspirated, washed in saline, streaked on slides and stained by the Papanicolaou technique. Cancer cells isolated by this technique are compared with a direct smear of the tumor (Fig. 2, 1). Cancer cell counts are determined by counting all the cancer cells present; the identical processing of each sample allows comparison.

RESULTS AND DISCUSSION

A total of 100 cancer patients and 11 control patients with non-malignant diseases were studied. The cases were classified in four groups: (1) gastro-intestinal cancers (esophagus, stomach, colorectal, gallbladder and pancreas), (2) genitourinary carcinomas (kidney, Wilm's tumor, bladder, urethra, and prostate), (3) breast cancer, and (4) miscellaneous malignancies (melanoma, ovary, cervix, lung, thyroid, and sarcoma).

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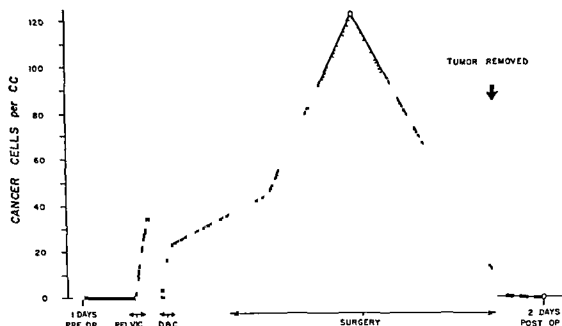


Fig 1 Cystadenocarcinoma of the ovary

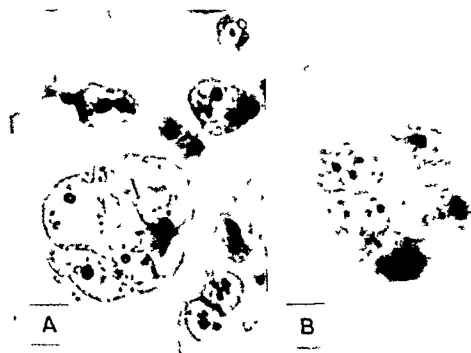


Fig 2 Cystadenocarcinoma of the ovary Papanicolaou stain ×1100 A Group of cancer cells forming acinar structure from peripheral blood during operation B Direct smear of resected tumor

CANCER CELLS IN THE BLOOD STREAM DURING RADICAL MASTECTOMY

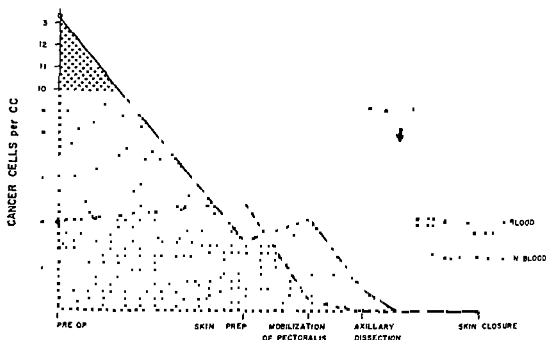


Fig. 3 Carcinoma simplex of the breast.

We have found the Papanicolaou technique far superior to other stains Although many large clumps of cancer cells were found (Fig. 6, 8), single cell identification of cancer cells was possible (Fig. 7). The cells identified as cancer cells were identical with those obtained by direct

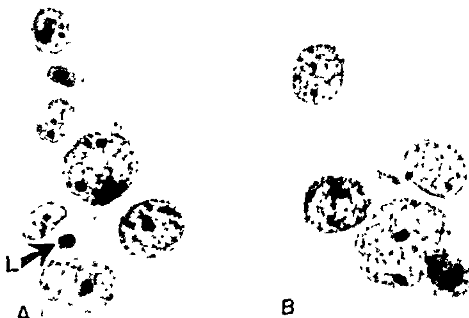


Fig 4 Carcinoma simplex of the breast. Papanicolaou stain, x1100 A Group of cancer cells from peripheral blood during skin preparation prior to surgery B Direct smear of resected tumor L = lymphocyte (After Cole *et al* Bull N Y Acad Med[7])

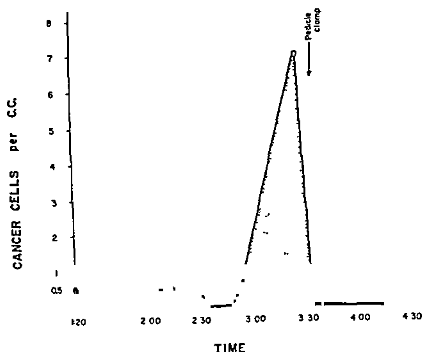


Fig 5 Carcinoma of the kidney



Fig 6 Ca of kidney, x500 Pap stain from catheter in inf vena cava during surgery

Fig 7 Carcinoma of kidney x1100 Papanicolaou stain from catheter in inferior vena cava during surgery





Fig 8 Ca of breast, x500 Pap stain from catheter in axillary vein during skin preparation for surgery

tumor smear, and confirmed by one of us, (EAM) a cytologist The positive cases included adenocarcinoma, transitional cell carcinoma, and malignant melanoma There appears to be a direct relationship between the advancing stage of the disease and the percentage of positive blood samples

Occurrence of Cancer Cells in the Blood. Of the entire group of patients 21 exhibited cancer cells in the blood stream (from any or all of the three venous sources) while no cancer cells were found in the control patients Of the 72 curable patients, 12 or 16 7% had cancer cells in the blood stream, while 9 of 28 or 31 1% of the incurable patients exhibited cancer cells *in the blood stream (Table 1)*

As seen in Table 2, twenty two cases of *curable gastrointestinal cancer* failed to show any cancer cells in the *peripheral blood,* while 2 of 9 cases showed cancer cells in the blood draining the tumor site In contrast, 5 of 17 *incurable gastrointestinal cancers* showed cancer cells in the peripheral blood Genitourinary, breast and miscellaneous cancers failed to show this difference This may indicate that the liver is a fairly effective filter of the cancer cells Of the incurable cases, 30 8% showed cancer cells in the peripheral blood as compared to 10 6% of the curable cases

*Table 1 Summary of 100 Cancer Patients**

(Blood samples obtained from any or all of three venous sources)

	CURABLE		INCURABLE	
	NUMBER	POSITIVE	NUMBER	POSITIVE
G I Tract	23	2	18	5
G U Tract	11	3	3	2
Breast	23	4	2	2
Misc (Melanoma Ovary etc)	15	2	5	1
Total	72	12 or 16 7%	28	9 or 31 1%

*62 patients were studied *during* a surgical procedure

Table 2 Analysis of Source of Positive Blood Samples†

	CURABLE			INCURABLE		
	PERIPHERAL	CATHETER	TUMOR SITE	PERIPHERAL	CATHETER	TUMOR SITE
G I Tract	0 (22)	0 (0)	2 (9)	5 (17)	0 (1)	1 (2)
G U Tract	2 (8)	2 (5)	1 (1)	0 (2)	1 (1)	0 (0)
Breast	3 (22)	2 (6)	0 (4)	2 (2)	0 (0)	0 (0)
Misc (Melanoma Ovary etc)	2 (14)	0 (1)	0 (2)	1 (5)	0 (0)	0 (0)
Total	7 (66) or 10 6%	4 (12)	3 (10)	8 (26) or 30.8%	1 (2)	1 (2)

†Figures in parenthesis ( ) represent number of cases studied

Role of Surgery Of the 62 patients studied during an operative procedure 12 or 19.3% exhibited cancer cells in the circulating blood (from any or all of three venous sources). Three of these positive cases gave adequate data for evaluating the role of surgical manipulation as illustrated in Figures 1, 3 and 5. In 2 cases, carcinoma of the ovary and carcinoma of the kidney (Fig. 1, 5) there appears to be a direct relationship between surgical manipulation and the number of cancer cells found in the circulating blood. In all 3 cases we failed to find cancer cells in the blood stream following the removal of the primary cancer. Figure 3 illustrates the simultaneous sampling of catheter and peripheral blood. In this case over twice as many cancer cells including larger clumps were found in the catheter sample (Fig. 8).

SUMMARY

From the data reported herein cancer cells have been demonstrated in the circulating blood (from any or all of three venous sources) in 16.7% of curable and 31.1% of incurable carcinomas. In 2 carefully studied cases there appears to be an increase in the number of cancer cells in the circulating blood incident to surgical manipulation. In certain cases it appears that the cancer cells disappear from the circulating blood soon (perhaps in a matter of minutes) after removal of the primary carcinoma.

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TUMOR CELLS IN THE BLOOD AND BODY CAVITY ASSOCIATED WITH MALIGNANCY OF THE LUNG AND GASTROINTESTINAL TRACT*

GEORGE E MOORE, AVERY A SANDBERG, EUGENE M BURKE
ROY T JOHNSON AND ALFRED D KATZ

Previous studies have established the presence of cancer cells in both the peripheral circulation and in the blood vessels draining tumor sites[3, 9] It is the purpose of this paper to analyze the occurrence of tumor cells in the circulation and in the body cavities according to the kind and relative stage of the malignancy

Tumor Cells in the Blood The method of preparing concentrated blood cell smears for the examination of tumor cells consists of complete, rapid sedimentation of the erythrocytes of a 5 cc. blood sample separate centrifugation of the plasma, and the preparation of thick smears from the resulting layer of packed cells

Microscopic examination of these cellular smears is done under low power Ordinarily, 3 slides are made from each blood sample and they are examined for a total of 80 minutes by two different people

Peripheral blood samples were obtained from the antecubital vein before operation Table 1 indicates the frequency of detection of tumor cells

It is noteworthy that no apparent increase in the frequency of tumor cells in the peripheral blood was found after surgery In contrast to this series of surgical patients, one of every three patients with advanced metastatic malignancy will have tumor cells in the blood[9]

Blood samples from veins directly draining the tumor site were obtained as soon as it was exposed and again immediately before completing excision of the specimen Whenever possible a vein site within 5 cm of the tumor was used and the 2 blood samples drawn from the same or adjacent veins The necessity of using different veins probably introduces a large sampling error and makes direct comparison of the frequency of tumor cells in the before and after surgical samples difficult The most awkward blood specimens to obtain were those from the lung Often the blood was taken from the pulmonary vein instead of segmental vessels

Table 2 is a summary of regional blood samples collected both before and after the surgical procedure It is interesting that in this small series the occurrence of tumor cells in both resectable lesions (curative procedures) and nonresectable lesions was similar (50%)

The influence of the kind of tumor upon the presence of tumor cells in the regional veins is also illustrated in this table It is not surprising to see that the relative frequency of tumor cells in the blood of regional veins seems to parallel the prognosis of these tumors The recovery of tumor cells from the pulmonary veins in 7 of 12 patients upon whom a curable operation was done is very disturbing

Table 4 is a comparison of the frequency of occurrence of tumor cells in regional veins before and after surgical manipulation There is an

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Supported in part by the Elsa D Jameson Memorial Fund and the Dorothy H and Lewis Rosenstiel Foundation

Table 1 Frequency of Tumor Cells in Peripheral Blood of Patients Having Malignancies of the Lung and Gastrointestinal Tract

	TOTAL CASES	TUMOR CELLS	NO TUMOR CELLS
Resectable Lesions	35	4*	31
Nonresectable Lesions**	29	15	14
Total	64	19	45

*Lung 1 Stomach 2 Colon 1
**Includes resections performed for palliation

increase in the number of cancer cells found in samples taken at the end of the operation on patients with colon and lung lesions Unfortunately the number of cases is small and the probable sampling errors too large to allow definite conclusions to be drawn

Table 2 Frequency of Tumor Cells in Regional Veins Draining Both Resectable and Nonresectable Lesions of the Lung and Gastrointestinal Tract

		RESECTABLE		NONRESECTABLE	
	TOTAL CASES	TUMOR CELLS	NO TUMOR CELLS	TUMOR CELLS	NO TUMOR CELLS
Lung	12	7	2	1	2
Stomach	17	7	3	4	3
Colon	29	8	15	4	2

The resectable malignancies are separated as to tumor type in Table 3 In this group there is a striking difference between the frequency of tumor cells found in patients with carcinoma of the colon and rectum (35%) and in lung and stomach (74%)

Table 3 Frequency of Tumor Cells in Regional Veins Draining Resectable Malignancies of the Lung and Gastrointestinal Tract

	TOTAL CASES	TUMOR CELLS	NO TUMOR CELLS
Lung	9	7	2
Stomach	10	7	3
Colon & Rectum	23	8	15

The resectable malignancies were also studied as a possible correlation between the presence of lymph node metastases and cancer cells in the blood In 21 patients with positive lymph nodes cancer cells were found in 13 and in 21 patients with uninvolved nodes 9 having positive blood samples

Tumor Cells in the Pleural and Peritoneal Cavities An assay of tumor cells in the body cavities was performed as soon as the chest was opened

Table 4 Comparison of the Presence of Tumor Cells in Regional Veins Before and After Surgical Manipulation

	+ BEFORE	+ AFTER	BOTH +	BOTH —
Stomach	7	8	4	5
Colon	4	11	2	18
Lung	3	6	1	4

and again upon termination of the surgical procedure Approximately 20 cc of normal saline was squirted along the body walls and viscera with a long asepto syringe and the fluid re aspirated from the cul de sac or most dependent area The fluid was centrifugated at 1 000 rpm and thick smears of the centrifugate made with a wire loop Both Papanicolaou and Wright Giemsa staining techniques were used The staining procedure was not an important factor influencing the diagnosis The slides were examined separately by a cytologist (E Burke) and a surgeon (G Moore) † and the results collated The method allows variation in techniques which resulted in sampling errors For example tumor cells might be present on both slides of one set but not in a second set prepared in a similar manner from the same patient A positive diagnosis was rarely made on the finding of a single cell Atypical cells were recorded as negative

The single cancer cells or clumps of cells were recognized by their large immature nucleus large nucleolus and staining properties The pathological diagnoses were not known to the screeners except for their own patients therefore patients with benign disease served as controls In this series of patients there were 6 5% false positive diagnoses This over reading should be considered when perusing Table 5 Differences in the exfoliation of cells by the different tumors were not remarkable The finding of cells in the peritoneal cavity in about 50% of all patients with colon stomach and lung cancer is impressive Even in curative patients there were cancer cells in 30% of the patients with lung cancer 65% of those with gastric cancer and 36% associated with carcinoma of the colon In the patients with nonresectable colon lesions the frequency of positive cells was 68%

An analysis of the presence of tumor cells before and at the end of the operative procedure is depicted in Table 5 There is no evidence that the operative procedure spread a detectable number of tumor cells

DISCUSSION

The success of excisional surgery for malignancy is limited by the frequency with which cancer cells are transported to distant sites The present studies confirm the presence of tumor cells in the blood and body cavities even in curable cases

The limitations of the techniques employed to find tumor cells in the blood should be kept in mind The volume of the blood samples is small and the opportunity for sampling errors large Further, the physical characteristics of the tumor cells such as size shape and specific gravity

†Dr J Badillo and Dr T Kondo performed preliminary screening

Table 5. Presence of Tumor Cells in Pleural and Peritoneal Cavities Before and After Surgical Manipulation in Carcinoma of Lung, Stomach and Colon

	+ BEFORE	+ AFTER	BOTH +	BOTH −
Stomach	8	9	7	8
Colon	18	16	14	41
Lung	10	5	5	14

vary considerably and significant numbers of cells are probably destroyed or lost by sedimentation with the erythrocytes. The latter statement has been true of comparable studies with experimental tumors.

Similarly, limitations exist in the study of the samples from the body cavities. The variety of cells, degenerating cells, and inflammatory cells, reduces the accuracy of diagnosis as is evident from 6.5% false positives in this study.

Prevention of cellular spread is difficult since it probably takes place continuously once the tumor has invaded blood vessels and lymphatics, and penetrated out into a serous cavity. The finding of tumor cells in the blood and serous cavities at the beginning of the operation is evidence contrary to the thesis that most tumor cells are spread by operative manipulation. Logically, it would be difficult to dismiss the importance of body movements, respiration, and peristalsis in effecting the spread of the cancer cell. Finally, the active amoebic movement of the cells themselves undoubtedly aids their entrance into blood vessels, vein, and tissue spaces.

Nevertheless, this investigation contains preliminary data indicating that surgical manipulation probably does increase the spread of tumor cells into the blood stream. This contrasts with the report of Engell[3] that no increase in the occurrence of tumor cells occurred in the regional veins of patients with colon carcinoma.

Other factors may influence the establishment of tumor cells and thus exaggerate the importance of the spread of tumor cells by surgery. These include the exposure of injured tissues to the cancer cells and a theoretical decrease in host resistance to malignancy as a result of the stress accompanying surgery. A recent experimental study of implantation by Goldie[4] is pertinent to the former proposition and indirect evidence of the latter has been reported by Pomeroy[8] and many others.

The finding of tumor cells floating in the blood draining tumor sites should not be surprising when one considers the frequency with which blood vessel invasion of blood has been in cancer of the lung, stomach, and colon. Johnson, *et al.*[5] have found blood vessel invasion in 70% of lung cancer. There was only a 6% five year survival in such patients whereas patients without blood vessel invasion had a 70% five year survival. In 1949, Meissner[7] reported that blood vessel invasion was present in 57% of resected gastric carcinomas. Sunderland[10] noted vein invasion by carcinoma of the rectum and sigmoid in 27.6% of the 210 resected specimens.

Our studies confirm and supplement to a remarkable degree these observations made by entirely different techniques. (Compare with Table 2.)

Cancer cells are not common in the peripheral blood of patients with "curable" lesions. Even in those patients having dozens of tumor cells in each smear preparation from regional vein blood, it may be impossible to find cells in the peripheral circulation. Undoubtedly, the vast majority of cancer cells are filtered out by the lung and liver and in terminal capillaries, and destroyed by the host. However, some cells and clumps of cells are able to enter the peripheral circulation by passing through arteriovenous shunts or by other mechanisms as yet unknown. The experimental work of Zeidman and Buss[12] is germane. If larger peripheral blood samples were collected and more effective methods of concentrating them could be devised, tumor cells might be found even in early cancer patients. It is doubtful, however, that the examination of blood for tumor cells could ever be a useful cancer detection procedure.

If it can be established that beyond doubt a *clinically significant* spread of tumor cells is associated with surgery, the importance of prevention by physical and chemical techniques is of utmost importance. These include: (1) early isolation and division of the veins draining all tumor sites;[1] (2) occlusion of the colon lumen on each site of the tumor; (3) the suggestion by many surgeons that the tumor bearing tissue be wrapped in an impermeable bag as soon as feasible; and (4) the well established principle of cancer surgery that care be exercised to minimize manipulation of the tumor.

As yet, there is no method of increasing host resistance to malignancy in humans; however, successful experiments in animals indicate this future possibility.

Much interest in cancer chemotherapy as an adjuvant to surgery has been stimulated by the reports of Cruz, McDonald, and Cole.[2] The success of this combination therapy depends upon the validity of many considerations discussed in this report. Unless a significant number of distant metastases are directly associated with surgery, the application of a compound with only a moderate cytolytic effective action only at the time of surgery would probably not be effective. Until the advent of extremely effective, specific chemotherapeutic agents, it would be unlikely that destruction of residual established tumor could be expected.

Keettel and Elkins[6] have documented the occurrence of cancer cells in the peritoneal cavity of patients with various gynecological malignancies. On the basis of these findings they have supplemented the surgical and x-ray treatment of ovarian malignancy with the intra-abdominal instillation of radioactive gold.

Shapiro[11] has shown experimentally that with a sensitive tumor and effective chemotherapeutic compound, the combined use of surgery and chemotherapy results in many more cures than the use of either singly.

Cooperative surgical adjuvant chemotherapy investigations in certain teaching hospitals and in the Veterans Hospitals have been established under the supervision of the Clinical Panel of the Cancer Chemotherapy National Service Center. It is hoped that these studies will provide a valid evaluation of adjuvant chemotherapy with exposure of a minimal number of patients to the toxic drugs presently available.

SUMMARY

1 The finding of tumor cells in the peripheral blood of patients with curable cancers of the lung stomach, and colon is rare The frequent occurrence of tumor cells in blood from veins directly draining cancers of the lung stomach and colon can be demonstrated

2 There is unverified evidence that the frequency of tumor cells in the regional veins is increased by surgical manipulation

3 Tumor cells can also be found in the body cavities of about one third of patients with resectable colon malignancies and two thirds of those with unresectable lesions There was no increase in the number of tumor cells found at the end of the operation

4 The use of cancer chemotherapy in conjunction with excisional surgery is suggested as a possible method for eradicating unestablished residual tumors spread beyond the confines of the surgical procedure

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Table 1 Results of Perfusion of Hind Limb and Midgut in the Dog

	NUMBER OF EXPERIMENTS	AGENT MG /KG	NUMBER OF SURVIVORS
Hind Limb	7	$\bar{0}$	6
	4	2 mg P A M	4
	7	3 5 mg P.A M	1
	2*	3 5 mg P.A M	0
S M A	4†	$\bar{0}$	4
	3‡	0 1 HN_2	1
	4	0 2 HN_2	2
	2	0 3 HN_2	0

*Phenylalanine Mustard direct intra arterial without perfusion
†Only 2 out of 24 survived while developing technique
‡2 deaths from causes unrelated to HN_2

received 0 2 mg /kg body weight and survived but all dogs receiving more than 0 2 mg died

At present we are unable to report on what is the safe dosage of nitrogen mustard that can be perfused through the isolated liver as this work has not been completed

DISCUSSION

It appears that the alkylating agents may be useful for perfusing malignant tumors that are regionally confined but not resectable In contrast to previous modes of administering these agents perfusion techniques developed in this study offer the following advantages (1) minimal toxic systemic effects (2) achievement of higher local concentrations of the agent (3) maintenance of high oxygen tension (4) administration of the agent under pressure assures dissemination throughout the vascular bed (5) residual unbound agent can be washed out of the isolated bed at the conclusion of treatment (6) longer acting agents remain in contact with tissue for the duration of their action shorter acting agents can be given in small repeated doses to maximum tolerance

When administered orally or intravenously the safe dose of phenyl alanine mustard is 2 mg /kg of body weight[5] Thus the 2 mg /kg of body weight which was administered to the hind limb of the dogs represents a very high concentration of the drug as the portion of the limb perfused only represents about 1/20th of the total body weight

Only one patient has been treated by a perfusion technique and while it is too early to make any statement on the eventual outcome of the case the early results have been promising This patient had metastatic melanoma in the dermal lymphatics of the left leg and thigh Since perfusion with phenylalanine mustard 4 months ago many of these lesions have disappeared and growth of others appears to have been arrested

SUMMARY

Selected perfusion of isolated viscera with chemotherapeutic agents using an extracorporeal circuit offers many advantages over conventional intravenous or intra arterial therapy The technique employed in perfusing

the hind limb, the midgut and the liver of dogs has been developed When given in a single dose in perfusing the hind limb of dogs, the safe dosage of phenylalanine mustard is 2 mg/kg of body weight In perfusing the superior mesenteric artery and vein with nitrogen mustard the safe dosage in dogs is 0 1 to 0 2 mg/kg of body weight

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THE ROLE OF CELLULAR DOSAGE ON "TAKES" FOLLOWING INOCULATION OF WALKER 256 TUMOR CELLS IN THE RAT*

ROBERT J OVERSTREET AND GERALD O MCDONALD

It is now well established by Engell,[1] Moore,[2] and workers in our laboratory,[3] that tumor emboli can be recovered from the peripheral blood of patients with cancer Some of these cells obviously produce metastases, but most presumably die or are killed by the host immunity before they can acquire vascularity to continue their growth However, little is known concerning the number of cells which can be tolerated before the host immunity is overpowered and metastases develop

We are, of course, well aware of the danger of metastases by implantation, but very little data is available to justify a statement regarding the likelihood of growth from a few cells inoculated intravenously compared with an equal number of cells inoculated subcutaneously and intraperitoneally

These series of experimental procedures were performed in an effort to determine the number of cells necessary to cause the growth of a tumor We also wished to determine if inoculation of cells by various routes would result in variation in incidence of growth

METHOD

The Walker 256 tumor was maintained in white, female Sprague Dawley rats Under aseptic operating room technique, a tumor cell suspension

*From the Department of Surgery University of Illinois College of Medicine Chicago Illinois Supported in part by grants from the Illinois Division of the American Cancer Society and the Chicago Community Trust (Rosa Kuhn Levy Fund)

was prepared by first finely mincing the tumor and adding 3 ml pooled plasma and 5 ml physiologic saline This mixture was passed through a fine stainless steel wire filter containing 80 perforations per linear inch The resulting suspension was made up almost entirely of single cells and contained only an occasional cellular clump The number of tumor cells per ml of suspension was determined by counting the cells in a hemocytometer using 1 2000 eosin, made up in Tyrode s solution, as the stain The unstained cells were counted, the stained cells being considered not viable The initial suspension then was diluted so that various portions contained 250, 500, 1000, 1500, and 2000 tumor cells

These different cell suspensions were injected into white female Sprague Dawley rats, weighing from 150 to 175 grams Four groups of animals were used consisting of 25 animals each

One group received the inoculation of the various strength suspensions via the portal vein It was necessary to anesthetize these animals with pentobarbital before performing a celiotomy for exposure of the portal vein

A second group (2), received subcutaneous inoculations of the tumor cells along the right flank after the hair had been shaved and the skin prepared with a 5% tincture of iodine solution and alcohol Group 3 received intraperitoneal inoculations of the cell suspensions, while Group 4 received intracardiac inoculations of the suspensions In each case, the skin was prepared as for the subcutaneous injections, the cells being injected through a fine needle into the peritoneal cavity or into the right heart, in the latter case passing the needle through the chest wall along the left lower border of the sternum

The entire procedure for each group of 100 animals was completed within 90 minutes after the tumor suspensions were prepared

All animals were examined post mortem for evidence of tumor and the percentage of "takes" for the various cell suspensions and the different methods of administration of the tumor suspensions were determined

RESULTS

In Table 1 are summarized the results following inoculation of the various dilutions of the cell suspensions Approximately 170 animals were injected with each dilution The incidence of growth following inoculation varied with the number of cells inoculated Two hundred fifty cells resulted in a smaller incidence of growth than did the other suspensions

Table 1 Growth According to the Number of Cells Injected

NUMBER OF ANIMALS	NUMBER CELLS INOCULATED	AVERAGE PERCENT TAKE
172	250	87.2
166	500	97 0
168	1000	92.2
161	1500	95 7
175	2000	98 3

Table 2. Growth According to the Site of Injection

NUMBER OF ANIMALS	SITE OF INJECTION	AVERAGE PERCENT TAKE
223	Intraperitoneal	88.3
225	Subcutaneous	94.2
199	Intraportal	96.0
195	Intracardiac	98.5

The incidence of growth according to the site of injections is listed in Table 2. The average per cent growth for the subcutaneous, intraportal, and intracardiac routes was approximately the same; however, the incidence of growth following intraperitoneal inoculation was somewhat less, averaging 88.3%.

The above results were recent, being obtained during the past 7 months. However, 18 months prior to that time, results obtained with counted cell suspensions of the 256 tumors were markedly different. At this earlier date, suspensions containing 150,000, 100,000, 50,000, 10,000, 5,000, and 1,000 cells were injected, each concentration being injected into 10 animals. One hundred fifty thousand cells and one hundred thousand cells, when injected, caused growth of tumor in 9 or 90% of each group of 10 animals. Inoculation of 50,000 cells resulted in growth in 3 or 30%, 10,000 in 1 or 10%, and 5,000 in 1 or 10%. No growth resulted in any of the 10 animals injected with 1000 cells.

DISCUSSION

Since the time of the initial scout experiments with counted cell suspensions of the 256 tumors, the virulence of this tumor in our laboratory has increased rather markedly. Now the tumor will cause growth in 87 to 98% of animals inoculated with cell suspensions containing 250 to 2000 tumor cells. However, at the time of the initial experiments, inoculation of 100,000 to 110,000 cells was necessary before similar incidence of tumor growth would result. One thousand cells caused no growth and five thousand cells resulted in growth in only one animal out of 10 inoculated with this number of the 256 tumor cells.

At the present time, there appears to be some decrease in the incidence of growth when 250 cells are inoculated as compared with the incidence when 500 to 2000 cells are used. The intraperitoneal route of administration appears to result in less growth of tumors with all cell strengths. The 88% incidence of growth in 223 animals inoculated intraperitoneally appears to be significantly less than the incidence occurring with the subcutaneous, intraportal or intravenous (intracardiac) routes. It, therefore, would appear that the number of cells inoculated and the place of inoculation influence the incidence of growth of the 256 tumor in the experimental animal. We have no evidence to indicate the number of cells actually necessary to insure tumor growth. Even if such data were available from our studies, the situation might not hold true with human tumors and we are well aware of the inadvisability of attempting to transfer such experimental animal results to humans.

SUMMARY

The 256 Walker tumor in our laboratory has undergone marked increase in virulence over the past 2 years, considerably fewer cells being required for growth now With the present tumor, growth in 87% to 98% of the animals resulted when 250 to 2000 cells were inoculated Prior to this time one hundred thousand to one hundred ten thousand cells were necessary in order to achieve similar results, no growth was obtained on injection of 1,000 cells and only an incidence of growth of 10% when 5,000 to 10,000 cells were inoculated There was a slight difference in "takes" with the number of tumor cells inoculated, 250 cells showing a slight difference when compared with results using larger numbers of cells The mode of administration also appeared to affect the incidence of growth, the intraperitoneal route of inoculation resulting in the smallest incidence This experimental data gives support to our assumption that not all cells desquamating from human tumors survive and produce metastases It is barely possible that the growth of disseminated cancer cells into metastases is related to the number of viable cells desquamated as the number of bacteria are related to the development of an infection in a host

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LIMITING FACTORS IN THE PROPHYLAXIS OF THE SPREAD OF CANCER AT OPERATION BY CHEMOTHERAPEUTIC METHODS*

C T McDONALD, J S HOWIE, P M WEEKS, AND C G THOMAS, JR

The recognition of the high incidence of tumor cell dissemination during the operative removal of malignant neoplasms has justified attempts toward prevention of further growth and spread [1] Although enhancement of host resistance is an attractive theoretical goal, practically, the chief efforts have been in the direction of prevention of cell dissemination, appropriate wound toilet following surgery and the use of chemotherapeutic and radiologic agents Experimentally, inhibition of transplantable neoplasms has been accomplished with both radiation[2] and cytotoxic drugs [3] This has led to the clinical trial of these agents on a prophylactic basis, with encouraging results in some circumstances However, because of the difficulties inherent in any clinical evaluation and in particular prophylaxis

*From the Department of Surgery University of North Carolina School of Medicine and North Carolina Memorial Hospital Chapel Hill North Carolina Supported in part by an Institutional Grant No 324 AME 13 29 from the American Cancer Society

against the spread of cancer, the general principles of therapy were thought better to be established in the experimental animal

The purpose of this investigation was to determine some of the factors that might limit such treatment of locally disseminated malignant neoplasms The study was so designed as to determine the importance of the "mass of the inoculum", the time of optimum susceptibility of the tumor and the optimum dosage as well as the best means of administration of the chemotherapeutic agent Since experimental tumors vary considerably in their susceptibility to chemotherapeutic agents,[4] a neoplasm was selected that had a known susceptibility to a specific agent

METHOD

Male and female mice of Strain A weighing between 18 and 22 gm were employed as hosts with the Ehrlich's mouse adenocarcinoma† in its ascites form as a test object Animals were maintained at a constant temperature, given water *ad lib* and provided a standard laboratory diet Ascitic fluid was removed from donor animals 7 to 12 days after tumor inoculation "Ascites tumor" for each experimental procedure was selected either from one donor or pooled from several donors Inoculations were performed through a #25 or #27 gauge needle with 0 2 cc of undiluted ascitic fluid Animals so inoculated developed ascites between the 4th and 5th day which was progressive and was associated with invasion of both the visceral and the parietal peritoneum Death usually occurred between the 12th and 18th day

Nitrogen mustard (Methylbis (Beta chloroethyl) amine) in a maximum dose of 2 5 mg /kg of body weight in a volume of 0 2 cc of distilled water was employed as the chemotherapeutic agent Evaluation of treatment was carried out by appraisal of the general appearance of the animal, presence of ascites changes in weight procured at 48 hour intervals and the time of death Necropsy was carried out in selected animals

Observations were made with regard to the following (1) effect of altering the time between inoculation and therapy with treatment being carried out at approximately 1 24 48, 72 and 96 hours (2) the effect of single versus divided or multiple treatments employing the same total amount of chemotherapeutic agent and (3) the effect of intravenous and intraperitoneal routes of administration of nitrogen mustard on intra peritoneally implanted tumor

RESULTS

1 **Effect of alteration in time between inoculation and treatment.** Initial studies were performed with 103 animals treated intraperitoneally at approximately 1, 24, 48, 72 and 96 hours after inoculation (Table 1) Although there was some variation in the pattern of survival, in general, those animals treated immediately or within 48 hours following inoculation had a higher survival rate without evidence of ascites than did those treated beyond 48 hours All animals treated after 96 hours or in whom there was already evidence of ascites at the time of treatment developed

†A slowly killing hypotetraploid ascites stock received from T S Hauschka in 1956

Table 1 Effect of Single Treatment of Nitrogen Mustard 4 2 5 mg/kg Administered Intraperitoneally on Growth of Ehrlichs Ascites Tumor (Evaluation at 28 Days)

TIME BETWEEN INOCULATION & TREATMENT	NO OF MICE	DEAD WITH ASCITES	ALIVE WITHOUT ASCITES
1 hour	21	5	17
24 hours	30	13	7
48 hours	34	16*	18
72 hours	28	21	7
Control	13	13	0

*2 died without evidence of ascites

progressive tumor although survival was prolonged by several days over that of controls

Necropsy studies revealed that survival seemed to be dependent upon institution of treatment before invasion had become evident histologically During the first 24 to 48 hours there was little evidence of invasion with the tumor apparently being located superficially on peritoneal surfaces At this time the neoplasm was apparently readily inhibited in its growth or destroyed Between 72 and 96 hours invasion was seen histologically and treatment with nitrogen mustard was apt to be followed only by prolongation of survival time

Necropsy of those animals surviving without evidence of ascites disclosed in some instances apparent nests of neoplastic cells infiltrating omentum and in particular tumor nodules (2 to 5 mm) at the site of the peritoneal puncture wounds The omental foci of tumor cells seemed to have little evidence of growth activity however the biological potential of these cells has not been tested by retransplantation

2 Effect of multiple treatments employing same total dose of nitrogen mustard The greatest survival without ascites as well as with the lowest incidence of subcutaneous tumors at injection sites was procured in those mice treated intraperitoneally at approximately 1 24 and 48 hours Initiation of identical treatment one day later resulted in two thirds of the

Table 2 Effect of Treatment with Intraperitoneal Nitrogen Mustard (8 mg/kg/day) Administered on Three Successive Days Evaluation at 16 Days

TYPE OF TREATMENT	NO OF MICE	DEAD WITH ASCITES	ALIVE WITHOUT TUMOR	ALIVE WITH ASCITES	ALIVE WITH SUBCUTANEOUS TUMOR
1 24 48 hrs	15	0*	6	2	6
24-48 72 hrs	15	0	8	2	6
48 72 96 hrs	15	3	1	8	6
72 96 120 hrs	1	13	0	2	

*1 died at 4 days without evident tumor

animals remaining alive without tumor. At 48 hours or more, however, few animals survived without any evidence of tumor. The greatest benefit observed in this latter group seemed to be the delay in the appearance of ascites as well as prolongation of survival time. Again, at necropsy, surviving animals without ascites disclosed nests of tumor cells in omentum and abdominal puncture wounds.

3. **Effect of intravenously administered nitrogen mustard on intraperitoneally transplanted tumors.** Following the transplantation of 0.2 cc. of ascitic tumor intraperitoneally one group of mice was treated with 2.5 mg. of nitrogen mustard per kilogram intravenously via tail vein at one hour and another at 24 hours. Those treated at one hour developed ascites which was delayed in its appearance to between the 6th an 10th day. Death, likewise, was prolonged to 18 to 22 days. Those mice treated at 24 hours after tumor inoculation with a single massive dose developed ascites and death by the 14th day. The beneficial results were quite inferior to a comparable dose intraperitoneally.

These observations suggested that a single intravenous treatment with nitrogen mustard was ineffectual as a curative procedure, but of some value in delaying death in those animals treated immediately. These poor results may in part be due to the apparent increase in toxicity of nitrogen mustard administered intravenously compared to the intraperitoneal route.

DISCUSSION

It is probable that the resistance of the host to any particular neoplastic transplant is primarily a quantitative phenomenon and that it may not be essential for every cell to be killed immediately for a host to survive. Thus, whereas the growth of a small inoculum can be successfully controlled, a larger mass of neoplastic cells may grow successfully.

Treatment at a time where there are relatively few malignant cells present may enable the host to control the remaining viable tumor. Regardless of the means of dissemination and the site of lodgment the neoplastic cells remain for a time as free grafts. Subsequent growth is dependent upon favorable environmental conditions. While in this somewhat precarious state of survival the neoplastic cell should be most susceptible to destruction by cytotoxic agents such as x-ray, chemicals and the like. Once the cells' integral relationship with the host has been re-established, it may be little more susceptible to therapy than the primary tumor. The findings in this study would support the thesis that the neoplastic cell in its free state is susceptible to an appropriate chemotherapeutic agent. Consequently, neoplastic cells introduced intraperitoneally can apparently be completely controlled with restoration of a normal life span of the animal if treatment is carried out before invasion has occurred. In keeping with this concept is the observation that residual tumor may appear and grow at sites of puncture wounds. These are areas that provide a better environment for growth and invasion

As might be anticipated from the action of nitrogen mustard, multiple doses appear to be more effective than single treatments. Since the most important action of mustard seems to be that of a nuclear as well as a cytoplasmic poison, repeated doses would have the theoretical advantage

of attacking those cells that are in mitosis and presumably unaffected by a single treatment. Ideally this might be accomplished within the time interval of one mitotic cycle.

Intraperitoneal administration of mustard is apparently better tolerated than a comparable intravenous dose. This also has the distinct advantage of providing a high local concentration with less effect systemically. Once invasion of peritoneum and abdominal viscera has occurred, however, it may be that control is better accomplished by way of the blood stream. It is realized that in general solid tumors are much less sensitive than identical tumors in their ascitic form.[5]

Although data gained from the laboratory animal cannot be transferred directly to the human, these findings would suggest at least some degree of prophylaxis against the growth of locally disseminated neoplastic cells in the human is reasonable and perhaps can be accomplished with an appropriate agent. For optimum results this agent should be employed as soon after dissemination as possible and before invasion of the host tissue has occurred. Multiple treatments would appear to be more effective than single treatments. Serosal surfaces are somewhat more resistant to invasion than raw surfaces and it is the latter that require the optimum methods of prophylaxis.

SUMMARY

The general principles of controlling the growth of locally disseminated human tumors have been approached by studying the Ehrlich's ascites tumor. This tumor is most susceptible to growth inhibition and death by nitrogen mustard when it is in its free state. Once invasion has occurred, its susceptibility decreases. The factors that limit the spread of these tumors in the experimental animal are probably similar to those that can be employed prophylactically in controlling the local dissemination of cancer in man.

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EFFECTS ON THE LIVER OF INTRAPORTAL ADMINISTRATION OF NITROGEN MUSTARD IN THE RABBIT*

WILLIAM J. GRABER III, HARRY J. MORESI, JR., AND EDWARD T. KREMENTZ

Previously published work indicates that local administration of nitrogen mustard by vascular perfusion of the involved organs may give more satisfactory results in the treatment of certain malignant tumors than total body exposure via peripheral vein administration.[1,2,3] This is thought to be due to the higher concentration of the drug coming in contact with the tumor which is made possible by this method. It has been suggested that hepatic malignancy, either primary or secondary, might be treated with favorable results by the direct intraportal administration of nitrogen mustard. However, the possibility of primary hepatotoxic effects from the intraportal administration of nitrogen mustard has been recognized and has been a cautioning factor in its clinical application. It is the purpose of this report to enlarge on work previously done concerning this problem and to present a histo-functional correlation which will justify and stimulate clinical trial of this concept.

METHOD

Twelve adult male rabbits were divided into 4 groups of 3 each and were given nitrogen mustard† dissolved in normal saline intraportally at the time of laparotomy. One group (B) was given 0.1 mg./kg. of body weight of nitrogen mustard. One group (C) 0.4 mg./kg. of body weight, and a final group (D) received 1.0 mg./kg. of body weight. The control group (A) was given normal saline intraportally. Liver biopsy and blood samples were taken on each rabbit at operation and served as controls for subsequent specimens. Blood samples were again taken on the 1st, 3rd, and 7th postoperative day, at the time of sacrifice. Also, at sacrifice, a section of portal vein and final liver specimen were obtained.

In addition a fifth group (E) of 2 rabbits were given 0.25 mg./kg. of body weight of nitrogen mustard each day for 4 consecutive days through a polyethylene tube which had been securely placed in a portal tributary and brought to the outside at the time of laparatomy. Besides the usual blood and liver control specimens, blood was taken on the 4th postoperative day (the day following the last injection) and at the time of sacrifice on the 10th postoperative day, at which time final liver and portal vein specimens were also obtained.

Determinations of thymol turbidity, zinc turbidity, and total bilirubin were performed on each blood sample. The cephalin flocculation test, performed in the control group of rabbits receiving saline only, increased, and as elevation in further serial tests would not have been significant, this test was discontinued. Sections of the liver and portal vein specimens were studied microscopically.

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*From the Department of Surgery, the School of Medicine, Tulane University of Louisiana Supported in part by the Cancer Teaching Grant CT 762 (C-8) from the National Cancer Institute of the National Institute of Health, Public Health Service.

Table 1. Values Obtained for Serial Liver Function Studies in Rabbits Given Nitrogen Mustard Intraportally. (Group A is the Control Group Receiving Normal Saline. B Received 0.1 mg., C 0.4 mg., and D 1.0 mg of Nitrogen Mustard, All per kg. of Body Weight.)

GROUP	RABBIT	DAY POST OP	CEPH. FLOC	THY. TUR UNITS	ZINC TUR UNITS	TOT BILIRUBIN (MG %)
A (normal saline)	1	0	1+	0.6	0.2	0.9
		1	2+	1.1	0.2	1.4
		3	3+	0.4	0.4	0.9
		7	3+	0.8	0.0	0.7
	2	0	1+	0.8	0.2	0.7
		1	1+	0.2	0.2	0.7
		3	3+	0.6	0.0	0.6
		7	3+	0.4	0.0	0.5
	3	0	2+	1.0	0.0	0.7
		1	3+	0.8	0.4	0.7
		3	3+	0.6	0.0	0.8
		7	3+	1.0	0.0	1.0
B (0.1 mg./kg.)	1	0		0.0	0.2	0.8
		1		0.8	0.8	0.8
		3		0.6	1.5	0.5
		7		1.0	0.4	0.3
	2	0		0.6	0.2	0.6
		1		0.2	0.0	0.6
		3		0.4	0.0	0.4
		7		0.6	0.0	0.9
	3	0		0.4	1.5	1.5
		1		0.2	0.6	0.6
		3		0.6	2.5	0.7
		7		0.2	1.1	0.9
C (0.4 mg./kg.)	1	0		0.4	0.4	0.5
		1		0.6	0.4	0.8
		3		0.8	0.0	1.5
		7		0.4	0.0	0.6
	2	0		0.4	0.2	0.5
		1		0.0	0.0	0.5
		3		0.0	0.0	0.5
		7		0.4	0.0	1.5
	3	0		0.8	0.0	0.6
		1		0.4	0.0	0.5
		3		0.4	0.2	0.5
		7		0.7	0.6	0.6
D (1.0 mg./kg.)	1	0		1.0	0.4	0.8
		1		0.6	0.8	0.3
		3		0.4	0.2	0.8
		7		0.4	0.2	0.6
	2	0		0.8	0.0	0.9
		1		0.4	0.0	0.4
		3		0.8	0.4	0.5
		7		0.8	0.2	0.6
	3	0		0.6	0.0	0.7
		1		0.4	0.2	0.7
		3		0.2	0.0	0.7
		7		1.3	0.2	1.0

Table 2 Values Obtained for Serial Liver Function Studies in Two Rabbits Given 0 25 mg /kg of Body Weight of Nitrogen Mustard on Four Consecutive Days Through an Indwelling Intraportal Catheter

RABBIT	DAY POST OP	THY TUR UNITS	ZINC TUR UNITS	TOT BIL MG %
1	0	1 1	0 4	0.2
	4	0 6	0 0	0 1
	10	0 6	0 0	0.3
2	0	0 8	0 0	0 1
	4	0 6	0 0	0 1
	10	0 6	0 4	0 4

RESULTS

By using the results of the liver function tests obtained in the control samples a set of normal values for these tests in the rabbit was developed They are as follows cephalin flocculation 0 to plus 2, thymol turbidity, 0 to 1 0 units, zinc turbidity, 0 to 0 4 units, and total bilirubin, up to 1 5 mg %

The results of the liver function tests of the samples taken from the first 4 groups (those receiving only one intraportal injection of nitrogen mustard and the control group) are presented in Table 1 The results from group E (those rabbits receiving four daily intraportal injections through an indwelling polyethylene catheter) are presented in Table 2

Results of the three serially performed liver function tests (Tables 1 and 2) indicate that no significant changes occurred regardless of the dose of nitrogen mustard administered In 3 instances the thymol turbidity exceeds 1 0 units the arbitrarily chosen upper limit of normal These were extremely slight elevations and are not thought to represent liver damage The zinc turbidity determinations were all within normal levels with the exception of the seventh postoperative day specimen in rabbit B 3 in which the zinc turbidity level was 2 5 units No explanation for this is apparent however, the other function tests on that specimen were within normal limits The determinations of total bilirubin showed no elevation above normal

Examination of the histological preparations of the liver specimens with the assistance of Dr Emanuel Farber, Associate Professor of Pathology, Tulane University, reflected the results of the liver function studies in that no damage to the hepatic parenchyma was demonstrated There was no evidence of hepatocellular necrosis fibrosis or metaplasia of any type seen in any of the specimens Photomicrographs of the liver taken at operation and again at sacrifice from one of the animals which received 0 4 mg /kg of body weight of nitrogen mustard are shown in Figure 1 Several specimens taken at sacrifice showed a definite reduction in the concentration of intracellular glycogen This finding was also observed at least minimally, in some of the animals in the control group It is probable that this reduction in intracellular glycogen is incidental to the

Table 1 Values Obtained for Serial Liver Function Studies in Rabbits Given Nitrogen Mustard Intraportally (Group A is the Control Group Receiving Normal Saline B Received 0 1 mg, C 0 4 mg, and D 1 0 mg of Nitrogen Mustard, All per kg of Body Weight)

GROUP	RABBIT	DAY POST OP	CEPH FLOC	THY TUR UNITS	ZINC TUR UNITS	TOT BILIRUBIN (MG %)
A (normal saline)	1	0	1+	0 6	0.2	0 9
		1	2+	1 1	0 2	1 4
		3	3+	0 4	0 4	0 9
		7	3+	0 8	0 0	0 7
	2	0	1+	0 8	0 2	0 7
		1	1+	0 2	0.2	0 7
		3	3+	0 6	0 0	0 6
		7	3+	0 4	0 0	0.5
	3	0	2+	1 0	0 0	0 7
		1	3+	0 8	0 4	0 7
		3	3+	0 6	0 0	0 8
		7	3+	1 0	0 0	1 0
B (0 1 mg /kg)	1	0		0 0	0 2	0 8
		1		0 8	0 8	0 8
		3		0 6	1.5	0.5
		7		1 0	0 4	0 3
	2	0		0 6	0.2	0 6
		1		0 2	0 0	0 6
		3		0 4	0 0	0 4
		7		0 6	0 0	0 9
	3	0		0 4	1.5	1 5
		1		0 2	0 6	0 6
		3		0 6	2 5	0 7
		7		0.2	1 1	0 9
C (0 4 mg /kg)	1	0		0 4	0 4	0 5
		1		0 6	0 4	0 8
		3		0 8	0 0	1.5
		7		0 4	0 0	0 6
	2	0		0 4	0.2	0.5
		1		0 0	0 0	0 5
		3		0 0	0 0	0.5
		7		0 4	0 0	1 5
	3	0		0 8	0 0	0 6
		1		0 4	0 0	0 5
		3		0 4	0.2	0.5
		7		0 7	0 6	0 6
D (1 0 mg /kg)	1	0		1 0	0 4	0 8
		1		0 6	0 8	0.3
		3		0 4	0 2	0 8
		7		0 4	0.2	0 6
	2	0		0 8	0 0	0 9
		1		0 4	0 0	0 4
		3		0 8	0 4	0.5
		7		0 8	0 2	0 6
	3	0		0 6	0 0	0 7
		1		0 4	0 2	0 7
		3		0.2	0 0	0 7
		7		1 3	0.2	1 0

Table 2. Values Obtained for Serial Liver Function Studies in Two Rabbits Given 0.25 mg./kg. of Body Weight of Nitrogen Mustard on Four Consecutive Days Through an Indwelling Intraportal Catheter

RABBIT	DAY POST-OP.	THY-TUR. UNITS	ZINC TUR. UNITS	TOT. BIL. MG. %
1	0	1.1	0.4	0.2
	4	0.6	0.0	0.1
	10	0.6	0.0	0.3
2	0	0.8	0.0	0.1
	4	0.6	0.0	0.1
	10	0.6	0.4	0.4

RESULTS

By using the results of the liver function tests obtained in the control samples, a set of normal values for these tests in the rabbit was developed. They are as follows: cephalin flocculation, 0 to plus 2; thymol turbidity, 0 to 1.0 units; zinc turbidity, 0 to 0.4 units; and total bilirubin, up to 1.5 mg. %.

The results of the liver function tests of the samples taken from the first 4 groups (those receiving only one intraportal injection of nitrogen mustard and the control group) are presented in Table 1. The results from group E (those rabbits receiving four daily intraportal injections through an indwelling polyethylene catheter) are presented in Table 2.

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of attacking those cells that are in mitosis and presumably unaffected by a single treatment Ideally this might be accomplished within the time interval of one mitotic cycle

Intraperitoneal administration of mustard is apparently better tolerated than a comparable intravenous dose This also has the distinct advantage of providing a high local concentration with less effect systemically Once invasion of peritoneum and abdominal viscera has occurred however it may be that control is better accomplished by way of the blood stream It is realized that in general solid tumors are much less sensitive than identical tumors in their ascitic form[5]

Although data gained from the laboratory animal cannot be transferred directly to the human these findings would suggest at least some degree of prophylaxis against the growth of locally disseminated neoplastic cells in the human is reasonable and perhaps can be accomplished with an appropriate agent For optimum results this agent should be employed as soon after dissemination as possible and before invasion of the host tissue has occurred Multiple treatments would appear to be more effective than single treatments Serosal surfaces are somewhat more resistant to invasion than raw surfaces and it is the latter that require the optimum methods of prophylaxis

SUMMARY

The general principles of controlling the growth of locally disseminated human tumors have been approached by studying the Ehrlich s ascites tumor This tumor is most susceptible to growth inhibition and death by nitrogen mustard when it is in its free state Once invasion has occurred its susceptibility decreases The factors that limit the spread of these tumors in the experimental animal are probably similar to those that can be employed prophylactically in controlling the local dissemination of cancer in man

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William J Graber III, Harry J Moresi, Jr, and
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METHOD

Twelve adult male rabbits were divided into 4 groups of 3 each and were given nitrogen mustard† dissolved in normal saline intraportally at the time of laparotomy One group (B) was given 0 1 mg /kg of body weight of nitrogen mustard One group (C) 0 4 mg /kg of body weight, and a final group (D) received 1 0 mg /kg of body weight The control group (A) was given normal saline intraportally Liver biopsy and blood samples were taken on each rabbit at operation and served as controls for subsequent specimens Blood samples were again taken on the 1st, 3rd, and 7th postoperative day, at the time of sacrifice Also, at sacrifice, a section of portal vein and final liver specimen were obtained

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		3	3+	0 4	0 4	0 9
		7	3+	0 8	0 0	0 7
	2	0	1+	0 8	0 2	0 7
		1	1+	0.2	0 2	0 7
		3	3+	0 6	0 0	0 6
		7	3+	0 4	0 0	0 5
	3	0	2+	1 0	0 0	0 7
		1	3+	0 8	0 4	0 7
		3	3+	0 6	0 0	0 8
		7	3+	1 0	0 0	1 0
B (0 1 mg /kg)	1	0		0 0	0.2	0 8
		1		0 8	0 8	0 8
		3		0 6	1 5	0 5
		7		1 0	0 4	0 3
	2	0		0 6	0 2	0 6
		1		0 2	0 0	0 6
		3		0 4	0 0	0 4
		7		0 6	0 0	0 9
	3	0		0 4	1 5	1.5
		1		0 2	0 6	0 6
		3		0 6	2 5	0 7
		7		0.2	1 1	0 9
C (0 4 mg /kg)	1	0		0 4	0 4	0 5
		1		0 6	0 4	0 8
		3		0 8	0 0	1 5
		7		0 4	0 0	0 6
	2	0		0 4	0 2	0 5
		1		0 0	0 0	0 5
		3		0 0	0 0	0 5
		7		0 4	0 0	1 5
	3	0		0 8	0 0	0 6
		1		0 4	0 0	0 5
		3		0 4	0 2	0 5
		7		0 7	0 6	0 6
D (1 0 mg /kg)	1	0		1 0	0 4	0 8
		1		0 6	0 8	0 3
		3		0 4	0.2	0 8
		7		0 4	0 2	0 6
	2	0		0 8	0 0	0 9
		1		0 4	0 0	0 4
		3		0 8	0 4	0 5
		7		0 8	0.2	0 6
	3	0		0 6	0 0	0 7
		1		0 4	0.2	0 7
		3		0.2	0 0	0 7
		7		1.3	0.2	1 0

Table 2. Values Obtained for Serial Liver Function Studies in Two Rabbits Given 0.25 mg./kg. of Body Weight of Nitrogen Mustard on Four Consecutive Days Through an Indwelling Intraportal Catheter

RABBIT	DAY POST-OP.	THY-TUR. UNITS	ZINC TUR. UNITS	TOT. BIL. MG. %
1	0	1.1	0.4	0.2
	4	0.6	0.0	0.1
	10	0.6	0.0	0.3
2	0	0.8	0.0	0.1
	4	0.6	0.0	0.1
	10	0.6	0.4	0.4

RESULTS

By using the results of the liver function tests obtained in the control samples, a set of normal values for these tests in the rabbit was developed. They are as follows: cephalin flocculation, 0 to plus 2; thymol turbidity, 0 to 1.0 units; zinc turbidity, 0 to 0.4 units; and total bilirubin, up to 1.5 mg. %.

The results of the liver function tests of the samples taken from the first 4 groups (those receiving only one intraportal injection of nitrogen mustard and the control group) are presented in Table 1. The results from group E (those rabbits receiving four daily intraportal injections through an indwelling polyethylene catheter) are presented in Table 2.

Results of the three serially performed liver function tests (Tables 1 and 2) indicate that no significant changes occurred regardless of the dose of nitrogen mustard administered. In 3 instances the thymol turbidity exceeds 1.0 units, the arbitrarily chosen upper limit of normal. These were extremely slight elevations and are not thought to represent liver damage. The zinc turbidity determinations were all within normal levels with the exception of the seventh postoperative day specimen in rabbit B-3, in which the zinc turbidity level was 2.5 units. No explanation for this is apparent, however, the other function tests on that specimen were within normal limits. The determinations of total bilirubin showed no elevation above normal.

Examination of the histological preparations of the liver specimens with the assistance of Dr. Emanuel Farber, Associate Professor of Pathology, Tulane University, reflected the results of the liver function studies in that no damage to the hepatic parenchyma was demonstrated. There was no evidence of hepatocellular necrosis, fibrosis, or metaplasia of any type seen in any of the specimens. Photomicrographs of the liver taken at operation and again at sacrifice from one of the animals which received 0.4 mg./kg. of body weight of nitrogen mustard are shown in Figure 1. Several specimens taken at sacrifice showed a definite reduction in the concentration of intracellular glycogen. This finding was also observed, at least minimally, in some of the animals in the control group. It is probable that this reduction in intracellular glycogen is incidental to the

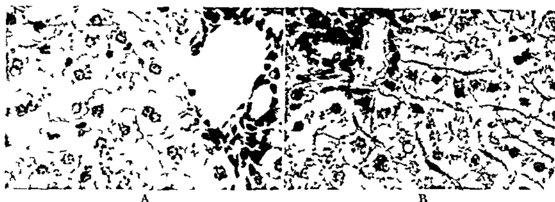


Fig 1 Photomicrographs of liver sections from a rabbit given intraportally 0 4 mg /kg of body weight of nitrogen mustard *A* is the operative specimen *B* is the specimen taken at sacrifice 7 days postoperatively (Hematoxylin Eosin stain) x 450

operation and recovery period and is not necessarily a result of the administered nitrogen mustard Studies of the sections of the portal vein taken at necropsy showed neither thrombus formation nor damage to the endothelium

DISCUSSION

It is the opinion of the authors that the tests of liver function and the histological appearance of the liver at a given time are not necessarily mutually compatible Therefore, any study of the possible hepatotoxic *effects of a drug, operative procedure, or disease should include both* anatomical and functional observations Because of this we feel that this present study, using both methods, presents a more accurate picture of the effect of intraportal nitrogen mustard on the liver and adds to the understanding of these phenomena previously described by Uram[5] *et al* who studied only histological changes

In reviewing the data collected in this study and considering the methods of testing for liver damage, it has become the opinion of the authors that the intraportal instillation of nitrogen mustard up to 1 0 mg /kg of body weight in the rabbit produces no resultant damage to the liver, either functionally or histologically In addition, there is neither intimal damage nor tendency toward thrombus formation in the portal vein

At present there is considerable interest in the use of intraportal nitrogen mustard as adjuvant therapy in the surgical treatment of abdominal malig nancy However, little information is available regarding possible toxic manifestations to the liver following this technique of administration The studies of McDonald *et al* indicate this therapeutic approach to be of real value[4] Warren Cole in personal communication indicated that sig nificant liver damage did not follow the clinical use of intraportal nitrogen mustard Our experimental findings support this observation

SUMMARY

1) Three groups of 3 rabbits each and a 4th group of 2 rabbits were given nitrogen mustard directly into the portal vein at operation in doses varying from 0 1 to 1 0 mg /kg of body weight The first 3 groups received a single direct intraportal injection, the 4th group received only saline intraportally

2) Blood and liver specimens were taken at operation, other blood samples were taken serially through the postoperative period, and final blood liver, and portal vein specimens were taken at sacrifice 7 days following injection of nitrogen mustard Thymol turbidity, zinc turbidity, and total bilirubin tests were performed and the tissue specimens were studied microscopically

3) No appreciable changes were noted in any of the postoperative liver function tests The histological sections of liver and portal vein showed no pathological changes

4) These observations indicate that the intraportal administration of nitrogen mustard up to 1 0 mg /kg of body weight in the rabbit is not injurious to the liver or portal vein

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TOXICITY OF NITROGEN MUSTARD IN RELATION TO OPERATIONS*

FRANCISCO MORALES AND GERALD O McDONALD

The known usefulness of nitrogen mustard in the treatment of cancer and the narrow range of safety between an effective dose and a toxic dose makes it essential to assemble as much data as possible concerning its toxicity Certain toxic manifestations such as bone marrow depression damage to the autonomic nervous system necrosing effect on tissues (unless used in dilute form) ulceration of the gastro intestinal mucosa[1 2] and hepatic necrosis[3] have been described after excessive dosage When used in therapeutic doses the only significant toxic effect on most *occasions is* a depression of the bone marrow After use of the conventional dose of 0 1 mg /kg of body weight on 4 successive days we have noted a significant depression of the bone marrow in over one half of our patients when the

*From the Department of Surgery University of Illinois College of Medicine Chicago Illinois Supported in part by the University of Illinois Graduate College and the Chicago Community Trust (Rosa Kuhn Levy Fund) and the American Cancer Society Illinois Division

drug is given at the time of operation, but in a much lower incidence when *given to patients not operated at the time of therapy*

In order to determine how much more toxicity is sustained when the drug is given at the time of operation than otherwise, we conducted animal experiments in which toxic manifestations following use of the drug at the time of operation were compared with those encountered when the drug was given to unoperated dogs

METHOD

Determinations of the white blood cell count, platelet count, Lee White clotting time, and heparin protamine titrations were obtained in the animals in the first 4 following groups Additional observations for vomiting, diarrhea, blood in the stools, wound infection, and weight loss were made At autopsy or time of sacrifice, sections of the liver and adrenals were obtained for microscopic examination

Group 1 (10 dogs). *To compare the effect of the operation alone upon* the changes in the formed elements of the blood and upon coagulation mechanisms, this group of animals was submitted to a standard operation after first obtaining control blood samples The operation consisted of cholecystectomy and splenectomy in order to simulate the stress load of operation in the human being

Group 2 (20 dogs). These animals were not subjected to surgery After *blood was obtained for the tests mentioned above, the animals were given* 0 3 mg nitrogen mustard per kilogram of body weight intravenously, this dose was repeated on three successive days Blood samples were obtained daily before each injection The animals surviving this large dose of nitrogen mustard were sacrificed on the 15th day following injection so that gross and microscopic observations of various organs could be performed Those animals dying during the first 15 days were subjected to post *mortem examinations*

Group 3 (16 dogs). These animals were subjected to the same operative procedure used in Group 2, namely cholecystectomy and splenectomy In addition, nitrogen mustard was administered, giving 0 1 mg of the drug per kilogram of body weight daily for 4 days This is the same dosage level we use clinically in our human series To simulate our methods used in the 'Prophylactic Treatment of Cancer',[4] the first dose of the *mustard (diluted in 250 cc normal saline) was injected into the peritoneal* cavity at the end of the operative procedure The subsequent doses were administered intravenously on the three following days

Group 4 (20 dogs). The procedure used in these animals was the same as that used in the Group 3 animals with the exception of the amount of nitrogen mustard administered In this group an amount equal to three times the human clinical dose was given (0 3 mgm per kilogram of body weight for 4 days)

Group 5 (24 dogs.) This group was included to obtain information concerning liver damage following injection of nitrogen mustard as determined by bromsulphalein retention and serum alkaline phosphatase levels The dogs were weighed and *divided into two subgroups* Group A (12 dogs) received 1 2 mg nitrogen mustard per kg of body weight in one dose, 6 received the drug intravenously and 6 intraperitoneally

The remaining 12 Group B received a single dose of 0.8 mg. of nitrogen mustard per kg. of body weight, 6 intravenously and 6 intraperitoneally. Control values were obtained before the drug was given and the tests were repeated at 3 day intervals until the 12th day at which time the animals were sacrificed.

RESULTS

As shown in Figure 1, maximum depression of the white count occurred in those animals receiving the larger dose of nitrogen mustard plus the operative stress. Eight (40%) of these animals died while only 4 animals (20%) died that had received the larger dose of mustard alone. There were no deaths in Group 1, those animals undergoing only the operative stress. There were 2 deaths (12.5%) in those animals having surgery plus the human clinical (0.1 mg./kg. body weight) dose of nitrogen mustard. All of the animals dying in these series expired between the 7th and 9th days which correlates with the period of greatest depression of the white counts. The animals dying showed evidence of overwhelming infection with generalized peritonitis and wound infection. There was no microscopic evidence of liver or adrenal damage beyond moderate vacuolation and fat deposition in the liver cells. All animals receiving nitrogen mustard showed anorexia, diarrhea and weight loss but no vomiting occurred and no blood was found in the stools.

Figure 2 demonstrates the platelet response in the 4 groups. There was no correlation between the time of death and the time of maximal platelet depression.

Equal prolongation of the clotting times occurred as shown in Figure 3 in those dogs receiving the larger dose of mustard plus surgery and in those receiving the larger dose alone. However there was no correlation

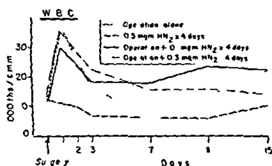


Fig 1

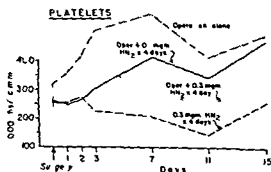


Fig 2

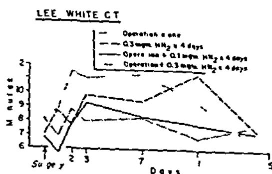


Fig 3

spontaneous tumor, and an adenocarcinoma and the results reported as negative,[2 3 4 5] the effect on embryonic liver by aminoguanidine indicated a need for testing it against a tumor of hepatic origin

It was also shown by Neuman and McCoy that when pyridoxal or pyridoxine was administered along with aminoguanidine sulfate, no effect on the embryonic liver was noted This suggests that an antagonist of pyridoxine might enhance the effect of aminoguanidine, accordingly desoxypyridoxine was selected for study as an adjuvant to aminoguanidine

METHOD

The tumor selected for study was hepatoma 98/15, transplanted into C_3H mice The animals were divided into groups as follows

Group 1. Five control and 5 treated mice of mixed sex were injected subcutaneously in the axillary region with 0 25 ml of a 50% solution of hepatoma cells in normal saline On the 28th day after transplantation of the tumor treatment was begun The treated animals were given 0 25 ml of a 4% solution of aminoguanidine sulfate daily and the controls were given 0 25 ml of normal saline daily Injections were made intraperitoneally and continued until the majority of the mice were dead

Group 2. Five control and 5 treated mice of mixed sex were used *The tumor was cut into pieces measuring approximately 3 mm*^{3} and injected subcutaneously into the axillary region by trochar Treatment was begun 2 days prior to transplantation Dosage, frequency, route of injection and duration of treatment were the same as for Group 1

Group 3. Ten control and 10 treated mice of mixed sex had tumors transplanted by the procedure described in Group 2 Treatment was begun on the day of transplantation The dosage, frequency, route of injection and duration of treatment were the same as for Groups 1 and 2

Group 4. Ten control and 10 treated mice of mixed sex received tumor transplants by the tumor fragment method described for Group 2 Treatment was begun the day after transplantation, the treated mice receiving 0 25 ml of a solution of 4% aminoguanidine sulfate and 0 076% desoxypyridoxine hydrochloride daily The controls were given 0 25 ml of normal saline daily The frequency, route of injection, and duration of treatment were the same as for Group 1

In each of the experiments the mice were divided into treated and control groups by random selection after the tumor transplantation The mice were weighed and the tumors measured in 3 dimensions every 3 days in every group

RESULTS

In Group 1 the average tumor size was 3 2 cc in the treated animals and 7 5 cc in the control animals after 43 days of growth The mean weight loss during this 43 day period was 3 8 gm in the treated animals and 4 5 gm in the control group

The average size of the treated tumors in Group 2 was 2 1 cc. as compared to a mean of 5 3 cc in the controls 36 days after transplantation Mean weight loss during this period was 3 6 gm in the treated animals and 3 1 gm in the controls Comparison of tumor growth rate in the individual mice of this group, during a treatment period of 23 days, is

Table 1 Tumor Size of Mice in Group 2 Arranged According to Size At First Measurement

	TUMOR SIZE OF FIRST MEASUREMENT (CC)	TUMOR SIZE AFTER 23 DAYS OF TREATMENT (CC)
Control	0	2.6
Treated	0	0.9
Control	0.1	5.3
Control	0.1	6.0
Treated	0.1	2.1
Treated	0.1	1.1
Treated	0.1	2.2
Control	0.4	5.2
Treated	0.4	2.9

shown in Table 1, tumors in treated and control mice which were of the same size at the first measurement are grouped together. One control mouse died before the 23rd day of treatment.

The mean tumor size in Group 3 was 1.6 cc. in the treated mice and 3.3 cc. in the control animals after 37 days of growth. Mean weight gain was 3.1 gm. in the treated as well as the control animals. This weight gain which differs from Groups 1 and 2 can be explained by the fact that the mice in these first 2 groups were fully mature when the experiment was begun, whereas the mice used in Groups 3 and 4 were young mice 4 to 6 weeks of age. At the end of this 37 day period there were 9 living treated animals and 6 living control animals.

Animals treated with aminoguanidine sulfate and desoxypyridoxine (Group 4) had a mean tumor size of 0.9 cc. as compared with a mean of 1.9 cc. in the control animals after 31 days of tumor growth. The mean weight gain in the treated animals during this period was 0.6 gm. and 0.5 gm. in the controls.

DISCUSSION

The results of these experiments indicate that embryonic inhibitors may have some use in cancer chemotherapy. Although the agent used in this experiment, aminoguanidine sulfate, did not cause complete inhibition of hepatoma growth, it did produce a decrease in the growth rate of the tumor. Initiation of treatment as late as the 28th day following tumor transplant gave as much adverse effect on the tumor as starting it at or soon before the transplants. The addition of desoxypyridoxine, a vitamin B_6 antagonist, did not increase the inhibitory effect of aminoguanidine.

The action of aminoguanidine sulfate on the hepatoma is not clear, however, the similarity of the structural formulas of this compound and arginine could indicate an antagonistic action against this amino acid.

Aminoguanidine alone appeared to have no significant toxic effect on the treated animals when given in the range of 500 mg./kg./day. Weight

losses or gains in treated mice were not significantly different from those in the control groups

Of particular interest to us was the fact that acute diarrhea resulted approximately 15 minutes after the daily injections of aminoguanidine sulfate into the treated mice which may indicate that it is rapidly metabolized In order to get a prolonged effect of the chemical it may be necessary to give smaller doses more frequently

Work on other guanidine derivatives both individually and in combination with other chemicals is now in progress in this laboratory

SUMMARY

Aminoguanidine sulfate an inhibitor of liver development in the chick embryo has been shown to inhibit the growth of hepatoma 98/15 in the C_3H mouse No greater decrease in hepatoma growth rate was noted when desoxypyridoxine was given with the aminoguanidine

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THE GROWTH OF HUMAN TUMORS IN HAMSTERS AFTER FREEZING ANOXIA AND HIBERNATION*

WILLIAM S FLETCHER AND W BRADFORD PATTERSON

Since the transplantation of human tissues and organs is rapidly becoming one of the dominant areas of surgical research the preservation of tissues pending transplantation has grown into a problem of major interest Furthermore the accidental transplantation of malignant tissues or tumor cells is recognized as a very significant factor in the treatment of cancer We have been studying a group of human malignant tumors transplanted into hamsters and have made certain observations on cell survival which appear to be pertinent to this subject

The *tumors studied are of varied* cell type and originated from a number of different tissues and organs These are all maintained by serial

*From the Department of Surgery Harvard Medical School the Fifth Surgical Service and the Sears Surgical Laboratory Boston City Hospital Aided by a grant from the American Cancer Society



Fig 1 Technic of cheek pouch transplantation

transplantation in the cheek pouch of the cortisone treated Golden hamster The tumors are transplanted by depositing a 2 mm fragment of tumor in the everted cheek pouch using a #16 trocar as illustrated in Figure 1

The results of the experiments to be described are summarized in Table 1

A. Resistance to Anoxia To study tumor survival after anoxia nine freshly excised tumors weighing 100 to 200 mg were left in sterile humidified Petri dishes for periods up to 72 hours Fragments of these tumors were reimplanted in other hamsters at intervals, and when growth occurred these tumors were excised for histologic confirmation As we have previously reported[1] all tumors survived at least 12 hours, while 3 tumors survived after 48 hours No tumors survived exposure to anoxia at room temperature for 72 hours

Table I Tumor Survival with Anoxia and Cold

TYPE CANCER	SURVIVAL TIME AT ROOM TEMP HRS	SURVIVAL IN HIBERNATING HAMSTERS	SURVIVAL AT —80°C
1 Epidermoid (Parotid)	48	Yes	Yes
2 Epidermoid (Penis)	48	—	—
3 Embryonal (Testis)	24	Yes	Yes
4 Epidermoid (Mouth)	24	—	—
5 Undifferentiated (Prostate)	12	—	No
6 Adenoacanthoma (Uterus)	24	Yes	Yes
7 Melanoma (Skin)	24	—	—
8 Adenocarcinoma (Uterus)	12	No	Yes
9 Undifferentiated (Thyroid)	48	Yes	Yes

B. Resistance to Hibernation (+5°C). To study the possibility of temporarily "storing" human tumors and to determine how growth would be affected by low temperatures, 5 of the above tumors were transplanted into hibernating hamsters.[2] Hamsters from 5 to 10 months of age were kept at 5°C ± 2°C until they hibernated. Tumor fragments were then implanted as previously described. The animals invariably awoke during the transplantation but usually returned promptly to hibernation. During the 6 weeks after transplantation they hibernated 40 to 96% of the time after which the hamsters were moved to the warm room and observed until the tumors either matured or regressed.

Tumor growth was markedly inhibited during hibernation and all but one grew normally thereafter. None of the tested tumors exceeded 0.2 cm in diameter in the 6 week period of hibernation whereas the tumors used would ordinarily grow to 0.4 to 1.0 cm in diameter in 2 to 4 weeks.

C. Resistance to Freezing (—80°C). Six of these tumors were frozen at —80°C. Healthy transplants were excised and minced in 5% dextrose and water, with or without added glycerol. The fragments were then placed in ampoules, sealed and placed directly in a freezer at —80°C. For reimplantation ampoules were thawed rapidly by immersion in a water bath at 37°C. Five of the 6 tumors tested have survived freezing, some for periods up to 6 months, and we are continuing further studies on this method of storage. Some of the tumors which will not survive freezing in 5% dextrose and water or in the "dry" state will survive if 20% glycerol[3] is used as a preservative.

Frozen tumors can again be serially transplanted and are histologically unchanged.

DISCUSSION

It is apparent from these observations that those human tumors which will transplant will withstand severe stresses and still retain their morphology and growth characteristics. It is interesting to note that all of these tumors came from patients who died of their cancer within a few months of the time of surgery. This resistance to treatment is reflected in the tumor's resistance to these various stresses.

That free tumor fragments will survive exposure to anoxia for as long as 48 hours points up a clinical danger and indicates that we should continue to make every effort not to disseminate tumor cells at operation.

The authors feel that freezing tumors and maintaining them in hibernating hamsters are feasible means of maintaining tumors for study at a later date without resorting to costly serial transplantation and possibly attenuating the tumor strain. Not all of the tumors may be successfully frozen by the same methods and it appears necessary to vary the technique somewhat for a given tumor. As shown by Craigie,[4] relatively slow freezing to —80°C and rapid thawing at 37°C are usually satisfactory methods of freezing.

CONCLUSIONS

In a group of human tumors which were serially transplantable in the cortisone treated Golden hamster, all of the 9 tumors tested survived after exposure to anoxia at room temperature for 12 to 48 hours.

Four of the five tumors tested grew at a reduced rate in the hibernating hamster and normally thereafter

Five of the 6 tumors tested survived freezing at —80°C

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THE EFFECT OF LOCAL INFILTRATION OF A HETEROLOGOUS ANTISERUM OF LYMPHOMA CUTIS IN MAN*

JAMES T GRACE FRANK GOLLAN W L TAYLOR, AND R I CARLSON

Renewed interest in the immunologic aspects of human cancer has been stimulated by recent experimental successes

In the mouse not only has active and passive immunity to several tumors been produced but therapeutic benefit has been observed with an anti leukemic serum [1 2]

This study represents the beginning of an investigation of immunity factors in human cancer It deals with a 42 year old man with lymphosarcoma of seven years duration He had generalized lymphadenopathy with marked skin involvement over the anterior chest and upper extremities He had previously received extensive radiation and chemotherapy

METHOD

Several involved lymph nodes were removed from the groin A 25% washed cell suspension in Ringer s solution was prepared This was combined with equal parts of Freund s adjuvant Several standard white laboratory rabbits were injected subcutaneously with 2 cc. of this emulsion Three weeks later a similar injection of the cell suspension alone was given After 2 weeks serum from the rabbits was obtained and pooled This constituted the antiserum used in the experiment

The *in vitro* effect of the antiserum on fresh tumor cells was studied with the phase microscope

Local infiltration of areas of skin involvement was used to evaluate the *in vivo* effect Several areas of clinically comparable skin involvement

*From the Surgical Service Thayer Veterans Administration Hospital and the Department of Surgery Vanderbilt University School of Medicine Nashville Tennessee Supported in part by a grant from the U S Public Health Service

were selected Representative portions of each were biopsied for histological comparison One area was chosen as a control and was not treated A second area was infiltrated with 2 cc of normal rabbit serum daily for 4 days The 3rd area was injected daily with 2 cc of antiserum for four doses At the end of this time all areas were centrally biopsied and microscopic sections obtained

RESULTS

Phase microscopy studies showed that fresh tumor cells brought in contact with the antiserum rapidly lost their motility and assumed a globular form cytoplasmic swelling ensued and rupture of the cell membrane occasionally followed Interestingly while the patient's red cells were rapidly lysed there was no apparent effect on the granulocytes

Microscopic changes were limited to the area treated with antiserum (Fig 1) Most notable was the framework stroma of histiocytes which appeared bare apparently due to partial or nearly complete dissolution of the lymphoid cells (as compared to previous section and sections from other two areas) The remaining lymphocytes were pyknotic Associated vascular changes were seen which suggested a hypersensitivity reaction

DISCUSSION

The immunologic approach to cancer is based on the premise that the cancer cell contains antigens not found in the normal cell This antigenic difference has been well established in various experimental tumors[3] Likewise Zilber studied a large number of human cancers using differential guinea pig anaphylaxis and demonstrated antigens in the cancer cells which were not present in normal cells from the same individual It appears that the cancer cell may contain normal cell antigens but the normal cell does not contain specific cancer antigens

In this study the whole tumor cell was injected into the rabbit for the production of the antiserum Doubtless many normal cellular antigens were included in the injection Secondly the whole antiserum was infiltrated into the skin lesion rather than the specific globulin fraction which had been freed of its antibodies to normal cell antigens This is no doubt accounts for the hypersensitivity reaction seen in the area treated with antiserum but absent in the area where normal serum had been injected

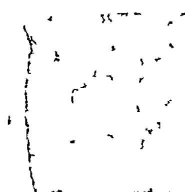


Fig 1 Area treated with antiserum

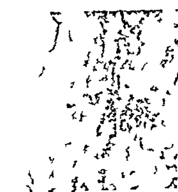


Fig 2 Area treated with normal rabbit serum



Fig 3 Untreated area

The apparent dissolution of lymphoid tumor cells is extremely interesting in that it suggests a local cytolytic action due to specific antibodies If this assumption is correct it represents important evidence of *in vivo* cytotoxicity of tumor antibodies in man

We are presently continuing these investigations Accurate definition of the cancer antigens and purification of the specific antibodies are the goals In addition cellular and humoral responses of cancer patients to various antigens are being studied with view to exploring the field of active immunity

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THE ACTION OF CERTAIN STYRYL QUINOLINE COMPOUNDS AGAINST WALKER 256 TUMOR*

F A DEPEYSTER AND P Y CHAN

In our continuing search for a more effective chemotherapeutic anticancer agent the favorable tumor depressing action of several orally administered styryl quinoline compounds reported by Bahner[2] and Lewis[3] and their associates attracted our attention They observed that 4 (p Dimethylaminostyryl) quinoline (4M20) and 4(p Dimethylaminostyryl) quinoline methiodide (4M2M) exhibited potent tumor depressing activity when administered orally against established rat tumors

Our study was undertaken to investigate the effect of these two styryl quinoline compounds (4M20 and 4M2M) when given *parenterally* against a *freshly disseminated* concentration of cancer cells We wished to evaluate the ability of these agents in protecting the host (rats) against lethal concentrations of cancer cells under conditions simulating actual modes of cancer dissemination encountered in clinical surgery

METHOD

Observing aseptic techniques from 16 to 24 female Sprague-Dawley rats weighing from 150 to 200 gm were anesthetized with intraperitoneal nembutal and operated upon at one time in a manner previously described by Cruz and associates[1] from our laboratory Alternate animals served as controls

Walkers 256 rat carcinosarcoma was chosen because of its hardy growth

*From the Department of Surgery University of Illinois College of Medicine Chicago
Aided by a grant from the Illinois Division of the American Cancer Society

rate when transplanted into animals, and minimal tendency for spontaneous regression This tumor has many of the characteristics of a carcinoma as well as a sarcoma A fresh saline suspension of the tumor which has been perpetuated in our laboratory for more than 2 years was prepared and counted after the method of Lucke[4] Concentrations of the fresh suspension made up with 110,000 and 10 000 cells/1 ml were injected under aseptic conditions into a large mesenteric vein or disseminated into the peritoneal or subcutaneous cavities as shown in Tables 1 to 5 The subcutaneous cavity was made by *blunt dissection through a 1 cm longitudinal skin* incision placed in the abdomino flank region Immediately after the cells were introduced into the rats, the anti tumor agent was dusted into the peritoneal or subcutaneous cavity and the wounds were closed with running cotton Rats receiving parenteral drug therapy were maintained on stand ard Purina rat chow with water *ad libitum* Thirty days following opera tion, all surviving animals were sacrificed and examined for the presence of tumor The anti tumor activity of these styryl quinoline compounds was judged by their ability to "protect rats against inoculated viable tumor cells which would kill the untreated (control) animals in 12 to 21 days

Table 1 Effect of Oral 4-(p Dimethylaminostyryl) Quinoline Against Intraportal 110,000 Walker 256 Tumor Cells

	NO RATS	% SURVIVED, WITHOUT TUMOR
Control	3	0
Treated	9	44
Dose 4M20 1 2 mg /day given by gavage 1 day before and for 7 days after intraportal tumor cell inoculation		

Table 2 Effect of Oral 4-(p Dimethylaminostyryl) Quinoline Methiodide Against 110,000 Intraportal Walker 256 Tumor Cells

	NO RATS	% SURVIVED WITHOUT TUMOR
Control	4	0
Treated	20	35
Dose 4M2M 0.3% in food 5 days before and 7 days after intraportal tumor cell inoculation		

Table 3 Effect of Intraperitoneal 4M20 Against 110,000 Intraportal Walker 256 Tumor Cells

	NO RATS	% SURVIVED WITHOUT TUMOR
Control	42	9.6
Treated	47	69.8
Dose 4M20 30 50 mg /kg dusted into the peritoneal cavity immediately after cell injection		

Table 4 Effect of Intraperitoneal 4M20 Against 10,000 Intraperitoneal Walker 256 Tumor Cells

	NO RATS	% SURVIVED WITHOUT TUMOR
Control	65	30 0
Treated	70	78.2
Dose 20 30 mg /kg dusted into peritoneal cavity immediately after cell dissemination		

Table 5 Effect of 4M20 Dusted Into Subcutaneous Cavity Containing 10,000 Walker 256 Tumor Cells

	NO RATS	% SURVIVED WITHOUT TUMOR
Control	20	5.3
Treated	20	88.9
Dose 4M20 20 30 mg /kg		

The anti tumor drugs used were 4 (p Dimethylaminostyryl) quinoline (4M20)† and 4(p Dimethylaminostyryl) quinoline methiodide (4M2M)†† provided through the courtesy of Doctor Carl Tabb Bahner and his associates

4M20 for gavage feeding was made by suspending the drug in 5% carboxyl methyl cellulose solution, so that 1 ml contained 1 to 2 mg 4M20 Rats received this preparation one day before, and daily, for 7 days following intraportal cell inoculation (Table 1) Water was given *ad libitum* Oral 4M2M was prepared by making a 0 3% mixture in finely ground Purina lab chow electrically mixed for one hour and fed 5 days before and 7 days after intraportal cell inoculation (Table 2) Water was given *ad libitum* 4M20 was given parenterally by dusting the powder into the peritoneal and subcutaneous cavities (30 to 50 mg /kg) using spatulas designed to hold 4 to 10 mg (Tables 3 to 5) A 4M20 acetate buffer solution was made up by dissolving 250 mg 4M20 in 25 ml of a standard acetate buffer solution (pH 4 7)

Toxicity studies were done on 8 dogs which received, intraperitoneally, 50 to 200 mg 4M20/kg , 4 rats were given 50 mg /kg 4M20 buffer solution intraperitoneally and 2 rabbits received 10 mg /kg 4M20 in a buffered solution intravenously The following serial laboratory determinations were done on the dogs over a period of 3 months red and white blood cell, hemoglobin and platelet counts Lee White coagulation time brom sulphalein (BSP) and heparin titrations Random urinalysis, NPN and total protein determinations were also done Only peripheral blood and platelet counts were done on the rats and rabbits

RESULTS

The results of this study are summarized in Tables 1 through 5 (4M20 and 4M2M) appeared to possess a degree of anti tumor activity offering

†(4M20) Prepared by Mr John N Fain Carson Neuman College Jefferson City Tennessee

††(4M2M) Prepared by Miss Joan Wilson Carson Neuman College Jefferson City Tennessee

some protection to rats from Walkers 256 tumor Animals receiving more than 20 mg 4M20/kg in powdered form lost from 10 to 30% of their weight A single intraperitoneal dose of 4M20 25 mg /kg in acetate buffer solution or intraperitoneal 4M20 60 mg /kg dusted into the peritoneal cavity would cause the death of the rat in about 5 weeks or sooner 4M20 (20 mg /kg) prepared in an acetate buffer solution seemed to have no significant anti tumor action in 45 rats against 10 000 cancer cells given intraperitoneally

A group of tumor bearing control rats was placed on a quantitative restricted diet to match the weight curve of the treated animals We concluded from this study that the anti tumor effect of 4M20 could not be attributed except to a minor degree either to a decrease in food con sumption or to an effect of the decreased rate of body weight gain *per se*

Where hepatic metastases were present in treated rats sacrificed at 30 days following intraportal cell inoculation the areas of growth were small discrete with minimal hemorrhagic ascites as compared to the overwhelming liver growth and large amount of bloody fluid in the control animals *Microscopically tumor from treated animals seemed to show a greater* sarcomatous element than observed in the controls

The 8 dogs which received from 50 to 200 mg 4M20/kg body weight intraperitoneally showed no significant evidence of toxicity by the lab oratory tests previously listed (including microscopic sections of the liver) under Methods except a 5 to 10% weight loss None of these animals died from effects of the drug Four rats which received 50 mg /kg 4M20 in acetate buffer solution intraperitoneally died in 4 days RBC WBC and platelet counts were depressed but returned to normal limits before death Temporary peripheral blood count depression was observed in one of the two rabbits which received 10 mg /kg 4M20 acetate buffer solution intravenously About 12% of the rats died on the table from hemorrhage excessive anesthesia or from causes undetermined and they are excluded from this study

The selection of 110 000 cells (Walker 256 tumor) for intraportal inocula tion was made based on work in our laboratory two years ago by Cruz and associates[1] They showed that this concentration of cells was near the minimum which would produce a high order of takes in the liver when injected into the portal circulation Since their initial work we have *recently observed 100% takes following* intraperitoneal injection of 10 000 to 5 000 cells and less These results may be due to the increased virulence of the tumor as well as to improved transplantation techniques Since we did not wish to overwhelm the rats with cancer the tumor dose was therefore reduced from 110 000 to 10 000 cells/ml in the more recent phases of this investigation (Tables 4 through 5)

Because of the relative insolubility of 4M20 we empirically dusted this agent into the rats peritoneal and subcutaneous cavities The lack of effect with 4M20 (20 mg /kg) prepared in an acetate buffer solution may have been due to inactivation by the buffer itself 4M2m is inactive against tumors except when mixed in the food (Bahner) therefore no further studies were carried out beyond the results shown in Table 2

Species variation coupled with the walling off of intraperitoneal 4M20

powder by the dogs omentum probably explained their high tolerance to this drug

This investigation was designed to simulate cancer spread under laboratory conditions suitable for determining the action of not only the styryl quinolines but other potential anti cancer agents as well We hope to extend the principles of this investigation to human trial as soon as a safe tumor dose is established

SUMMARY

Moderate anti tumor (protective) activity of certain styryl quinoline compounds (4M20 and 4M2M) was observed when given to rats following intravenous, intraperitoneal and subcutaneous inoculation of Walker 256 tumor cells

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UPTAKE OF 2 C 14 LABELLED TRYPTOPHANE BY MALIGNANT CARCINOID TUMOR*

JACK W COLE AND LEROY MATTHEWS

A syndrome characterized by diarrhea cutaneous flushing respiratory distress and valvular disease of the heart is frequently noted in patients with malignant carcinoid tumors[1] Lembeck[2] in 1953 demonstrated the presence of large quantities of 5 hydroxytryptamine (serotonin) in these tumors which probably explains these clinical manifestations

Udenfriend and Titus[3] have shown that the essential amino acid tryptophane is the precursor of serotonin and that in patients with malignant carcinoid tumors ingested tryptophane is diverted to the tumor tissue and excreted in the urine as 5 hydroxyindoleacetic acid

Indirect evidence for this pathway was afforded by the studies of Udenfriend Isotopically labelled tryptophane following administration to pa

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With the assistance of Mr Jack Krohmer

Table 1. 2 C-14 DL Tryptophane Uptake

TISSUE	COUNTS PER MINUTE/MG DRY WT
Carcinoid Tumor	10.2
Skeletal Muscle	1 5
Liver	6 8
Intestinal Mucosa	29 0

tients with carcinoid tumors, appeared in the urine as radioactive 5 hydroxyindoleacetic acid, the major metabolite of serotonin[4,5]

Studies in our laboratory provide additional direct evidence for this pathway.

METHOD

Twenty five microcuries of 2 C-14 DL tryptophane (Tracerlab) were given orally *to a patient with known malignant carcinoid, 5 hours prior to* surgery. At surgery biopsies of the metastatic carcinoid tumor, normal liver, skeletal muscle and small intestinal mucosa were obtained. The biopsies were placed immediately in a deep freeze unit The tissue was later oxidized and the resultant CO_2 assayed for C-14 content utilizing a flow type Geiger counter.

RESULTS

The results demonstrate the direct uptake of the ingested radioactive tryptophane by the metastatic tumor. (Table 1) Although the uptake by the tumor was less than that found in the intestinal mucosa it was significantly higher than that noted in normal liver or skeletal muscle. The high mucosal count may be erroneous in the light of the fact that surgery in the patient studied was undertaken for relief of a partial small bowel obstruction The possible interference with the transport of the tryptophane across the mucous membrane must be borne in mind

SUMMARY

Direct uptake of orally administered C 14 tryptophane by metastatic carcinoid tumor in a patient has been demonstrated and affords additional evidence of utilization by these tumors of tryptophane in the endogenous production of 5 hydroxytryptomine (serotonin)

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EXPERIMENTAL CANCER OF THE GASTROINTESTINAL TRACT*

A Preliminary Report

JOSEPH G FORTNER

Solution of the problems concerned with the etiology, pathogenesis, growth, and chemotherapeutic control of gastrointestinal cancer in humans depends, in great measure, on observations made in experimental animals. This work has been hampered previously by the marked resistance of the gastrointestinal tract of laboratory animals to carcinogenic agents and by the rarity with which spontaneous cancers affect this system.[9] The common occurrence of spontaneous tumors in the gastrointestinal tract and its appendages as well as adrenal, ovarian, and other tumors in a colony of Syrian (Golden) hamsters is therefore of considerable interest. The findings herein described were incidentally observed during experiments primarily concerned with evaluating the carcinogenicity of human bile.[2] Of added significance is the fact that some of these tumors have been successfully transplanted.

METHOD

This report concerns what is considered a representative sample of the 620 Syrian hamsters obtained as weanlings from a commercial dealer during the period of December 1954 to April 1955. Upon receipt from the hamstery, the animals were grouped into those merely observed and those injected subcutaneously with distilled water, saline bicarbonate solution, sodium deoxycholate solution, sesamine oil, cholesterol in sesamine oil, ox bile or human bile. Maintained under ordinary laboratory conditions, the animals were allowed to live out their normal life span. At death, complete necropsy and microscopic studies were done on each animal. Details of the experimental design and results are not reported now due to time limitations. However, the findings in different test groups are qualitatively similar but a variation in tumor incidence and in latent period for development of the tumors is present. A complete report will be subsequently published.

RESULTS

Preliminary observations have been made on 292 animals dying from 251 to 881 days after initiation of the experiment. One hundred seven or 36.5% of these animals were found to have tumors. 69 animals or 23% of the group had malignant tumors, 26 or 9% had only benign polyposis of some gastrointestinal segment and 12 or 4% had other benign tumors. Cystadenomas and other cysts of the liver, sebaceous gland adenomas, cellular blue nevi and hemangiomas of the spleen are representative of those benign tumors which were frequent but not included in any of the statistics.

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*From the Department of Surgery and the Andre and Bella Meyer Laboratories of Sloan Kettering Institute, Memorial Center, New York. Supported by grants from the National Institutes of Health, U S Public Health Service.

With the invaluable assistance and support of Drs A C Allen, H T Randall and I S Ravdin and the technical assistance of Miss Alice Gale.

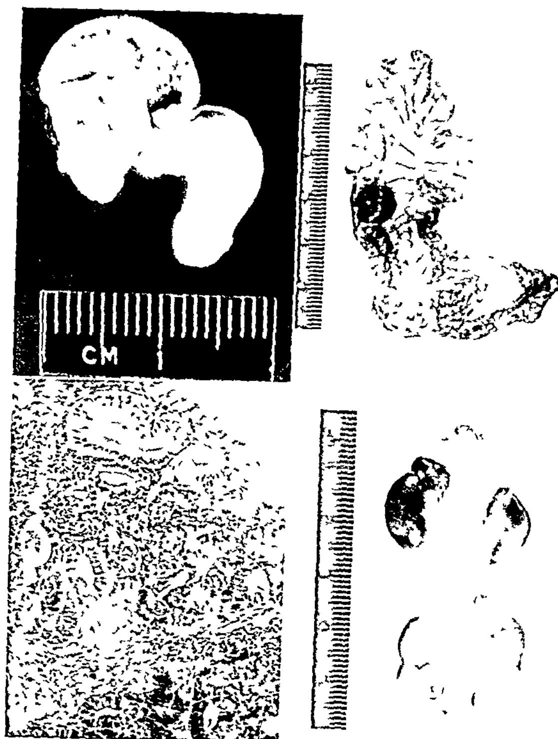


FIG 1 *(upper left)* Gross appearance of an ulcerating carcinoma of the hind stomach found in an animal dying 645 days after initiation of the experiment An adrenal cortical carcinoma was also present

FIG 2 *(upper right)* Gross appearance of an adenomatous polyp of the caecum observed in an animal dying 637 days after initiation of the experiment Malignant change was discovered microscopically

FIG 3 *(lower left)* Photomicrograph of an adenocarcinoma of the colon found in an animal dying 826 days after initiation of the experiment

FIG 4 *(lower right)* Gross appearance of an adenomatous polyp of the colon adrenal cortical carcinoma and small testes of an animal dying 654 days after initiation of the experiment

Primary adenocarcinomas of the glandular stomach, (Figure 1, page 194) small intestine or large intestine, (Figures 2 and 3, page 194) were found in 28 animals Benign polyposis of the intestine was associated with some of these cancers *An additional 10 animals had adenomatous polyps involving* some portion of the gastrointestinal tract but predominantly in the colon A leiomyosarcoma of the colon was present in 2, and an angiosarcoma of the colon in 1 animal

The other tumors observed are of possible importance in defining the etiology of the gastrointestinal tumors Adrenal cortical carcinomas were found in 7, adrenal cortical adenomas in 8 along with numerous instances of adrenal cortical hyperplasia There were cancers of the ovary in 7, cancers of the endometrium in 3, leiomyomas of the uterus in 2, and bile duct or hepatic cell cancers of the liver in 10 animals Cancers of the kidney and of the pancreas were each found in 2 animals Islet cell adenomas and lymphosarcomas were present in 3 and 8 animals respectively Malignant melanomas originated in 8, cancers of the thyroid in 2 and of the lung in 3 animals Two or more different primary tumors were found in 10% of tumor bearing animals No neoplasms of the pituitary glands were noted Study of cell types and distribution is in progress

With rare exception autopsied animals also had many non neoplastic *lesions Ulcerations of the gastrointestinal tract were frequent* The kidneys were involved commonly by some degree of amyloid nephrosis and nephrocalcinosis Vascular lesions consisted of hyaline necrosis of arteriols, thrombosis of peripheral vessels and of the cardiac auricular appendage, endocardial alterations myocardial necrosis and fibrosis as well as peri vascular inflammation Subcutaneous edema, pleural effusion and ascites were seen The liver demonstrated alterations consisting of central and periportal vascular inflammation and amyloid deposition, bile duct proliferation, cyst formation, increased hepatic cellular glycogen or fat accu mulation and multiple focal areas of necrosis Fat was frequently deposited in the pancreatic acinar cells Lipid and/or amyloid replaced, to varying degrees, the cells of the zonas reticularis and fasciculata of the adrenal cortex Ovarian luteinization and testicular atrophy were commonly pre sent *in animals of about 10 months of age or older Many of these lesions* resembled those characteristic of the General Adaptation Syndrome[8]

DISCUSSION

The frequent occurrence of spontaneous gastrointestinal neoplasms in a laboratory animal appears to be a new observation of potential im portance to the study of this form of cancer in man It provides opportunity for evaluating combined surgical and chemical treatment of gastrointestinal cancers under controlled laboratory conditions using the hamster tumors either in their *original or in transplant forms* Methods of preventing the development of gastrointestinal cancers can be studied There is afforded further means of study of the chemical and biologic changes which accom pany the development of adenomatous polyps and their conversion to adenocarcinomas New insight may be gained into the various unsolved problems concerned with the mechanics of metastatic spread, as well as of the chemical properties of tumors which metastasize As concerns the etiology and pathogenesis of gastrointestinal cancer, evaluation of the

experimental data available thus far suggests the possibility that the neo plasms of the gastrointestinal tract as well as of other sites might be caused by the same etiologic factors An unsuspected dietary deficiency or toxic agent may have initiated the carcinogenic process

Hormonal imbalance probably had some part in the development of the tumors for many lesions are of the type which have been induced by hormonal alterations (Figure 4, page 194) Adrenocortical carcinomas, adenomas, hyperplasia or marked amyloid replacement were almost invariably present These adrenal lesions have been previously found to develop in castrated hamsters[10] Testicular atrophy and ovarian lutienization or tumor formation were common Hyperplasia of the vagina and endometrium, endometrial carcinomas, uterine leiomyomas, lymphosarcomas, liver and kidney tumors are other examples of lesions which might be indicative of the hormonal imbalance In the evaluation of various possible pathogenic factors, it would seem important to bear in mind the demonstrated unique response of some viscera of the hamster to hormones The induction of kidney[5,6] and liver[7] tumors by estrogen administration and the failure of testosterone to inhibit the formation of stilbestrol induced uterine leiomyomas[1] are examples of this phenomenon

It is noteworthy that all neoplasms were of glandular type, of an endocrine organ or of a tissue previously demonstrated to be susceptible to hormone induced neoplasia in the hamster or other animal species A possible exception to this generalization is the malignant melanomas Although bronchial adenocarcinomas were present, squamous carcinomas of the bronchus have not been found Squamous carcinomas of the skin, fore stomach, urinary bladder, rectum, etc, were absent

CONCLUSIONS

Adenomatous polyps, adenocarcinomas, leiomyosarcomas and an angio sarcoma of the gastrointestinal tract have been observed to arise spontaneously in the Syrian hamster. A hormonal imbalance may be the etiological basis for the development of the neoplasms of the gastrointestinal tract as well as of other tissues With a possible exception, tumors observed in the animals of this experiment have been of glandular type, of an endocrine organ or of a tissue previously demonstrated to be susceptible to hormone induced neoplasia

An ideal experimental tool would now seem available for study of the various problems which are concerned particularly with gastrointestinal cancer Many spontaneous hamster tumors including intestinal are growing in active transplant form and are available to interested investigators

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Gastric Physiology

DUMPING SYNDROME REPRODUCIBILITY OF THE CLINICAL AND LABORATORY PHENOMENA IN ANIMALS AND IN NORMAL AND GASTRECTOMIZED PATIENTS*

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The post gastrectomy dumping syndrome has been surrounded by much mystery and confusion, probably due to a lack of agreement as to what constitutes this syndrome We recognize the dumping syndrome as a *symptom complex which is induced in many gastrectomized patients by* the ingestion of certain food substances, notably carbohydrates, and which has intestinal and vasomotor manifestations The intestinal symptoms include hyperperistalsis, bloating, epigastric discomfort, mild cramps, nausea, and frequently diarrhea The vasomotor symptoms include weakness dizziness, pallor, sweating, the desire to lie down, tachycardia, palpitation, and evidence of a reduced cardiac output

The studies of Roberts, Randall and Farr[1], Machella[2], and Fisher, Taylor, and Cannon[1] have indicated the importance of hyperosmolar materials rapidly entering the jejunum in initiating the phenomena associated with "dumping" The present investigation was undertaken to confirm and extend these observations

METHOD

A In the basic investigation of the clinical material, 31 patients were studied 6 weeks to 12 years after gastric resection Of these, 21 had hemi gastrectomy and vagotomy, 7 had more extensive partial resection, and 3 had total gastrectomy with Roux Y esophagojejunostomy These patients were all studied with an oral test of 150 cc of 50% glucose

B The second half of the study included patients and animals Five patients from the first group who had not responded to 150 cc of 50% glucose were retested at a later date with 300 cc of 50% glucose Ten persons with normal stomachs had a tube advanced through the pylorus into the duodenum or upper jejunum, 200 to 300 cc of 50% glucose were injected rapidly into the bowel through the tube In addition, 5 normal controls drank 150 cc of 50% glucose and another 5 normals drank 300 cc of 50% glucose

In 28 mongrel dogs, the spleen was removed and the jejunum divided just distal to the ligament of Treitz The distal end of jejunum was exteriorized and the proximal end was re anastomosed 18 inches distal to the point of division to form a Roux Y jejunostomy After recovery from operation the animals were tested under light pentobarbital anesthesia as follows 12 animals had 50 cc of 50% glucose injected directly into the

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jejunum, 13 animals had 150 cc of 50% glucose injected, and 3 animals had 300 cc of 50% glucose injected.

All tests were performed in the fasting state. Measurements of blood volume, hematocrit, hemoglobin, blood sugar, serum sodium, serum potassium, and serum chloride were made prior to ingestion of the test meal and at 5, 15, 30, 45, and sometimes 60 minutes afterwards. Clinical observations included recording of the blood pressure, pulse rate, respiratory rate, and symptoms. Electrocardiograms were recorded during the test on 18 of the patients. Blood sugar and electrolyte levels were not measured in the animals.

Blood volumes were determined by the use of radioactive iodinated serum albumin (RISA). The counts were made in a well scintillation counter with an anthracene crystal and fed into a scaler. Sugars were determined by the Folin-Wu colorimetric method, chlorides by the Volhard method, and sodiums and potassiums by flame spectrophotometry. Hemoglobins were determined colorimetrically and hematocrits by the Wintrobe method. The severity of the symptoms was graded in the manner suggested by Fisher, Taylor, and Cannon.[1]

RESULTS

A. The important data on these 31 patients are summarized in Table 1. There were no significant alterations of the serum electrolytes. The blood sugar determinations revealed normal glucose tolerance curves. Electrocardiograms were performed on 18 patients, 15 of whom showed the changes previously noted by Randall *et al.*[3]

Table 1. Type of Operation, Severity of Symptoms and Blood Volume Changes in the 31 Patients Tested with a Test Meal of 150 cc of 50% Glucose

TYPE OF OPERATION	NO. OF PATIENTS	HISTORY DUMPING	NO. WITH TEST SX	SEVERITY TEST SYMPTOMS	NO. WITH B.V. CHANGE	RANGE OF B.V. CHANGE
Hemigastrectomy Vagotomy	21	10	13	0 —8 1 plus—4 2 plus—3 3 plus—3 4 plus—3	12	200-1896 cc 6-34.5%
Subtotal Gastrectomy without Vagotomy	7	5	7	0 —0 1 plus—2 2 plus—0 3 plus—3 4 plus—2	6	528-2495 cc 9-32%
Total Gastrectomy	3	2	2	0 —1 4 plus—2	3	580-2249 cc 11-24%
TOTALS	31	17	22	0 —9 1 plus—6 2 plus—3 3 plus—6 4 plus—7	21	200-2495 cc 6-34.5%

Table 2 Severity of Symptoms and Blood Volume Changes in the Controls Intubated and Retested Individuals

TYPE PT	NUMBER OF PATIENTS	HISTORY OF DUMPING	NO WITH TEST SYMPTOMS	SEVERITY TEST SYMPTOMS	NO WITH B V CHANGE	RANGE OF B V CHANGE
Control 150 cc D50W Orally	5	0	0	0 0 0 0 0	1	340 cc. (5%)
Control 300 cc D50W Orally	5	0	1	0 0 0 0 ++	3	285 725 cc (5 10%)
Jejunal Tube 200 300 cc D50W	10	0	10	++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	10	170† 1570 cc (7 17%)
Gastrectomy Previously Tested 300 cc D50W Orally	5	0	5	++++ ++++ ++++ ++++ ++++	5	260†† 2466 cc (4 33%)

†Patient vomited 350 cc 5 after injection of glucose
††Major symptoms occurred about 20 after end of test
No blood volume obtained at this time

Table 3 Blood Volume Changes in Animals with Various Volumes of 50% Glucose

TEST MEAL	NO DOGS	NO WITH B V DECREASE	AVERAGE B V DECREASE
50 cc D50W	12	3	8 7% (5 14%)
150 cc D50W	13	13	18% (7 31%)
300 cc D50W	3†	3	18% (13 27%)

†2 Deaths

B The important data on the retested gastrectomized patients and the normal patients studied either with oral ingestion or jejunal instillation of the 50% glucose solution are summarized in Table 2 The blood chemical determinations again failed to reveal any important changes Electrocardiograms were not done in these individuals

The important data on the animal experiments are summarized in Table 3 Blood chemistries and electrocardiograms were not performed on the animals

DISCUSSION

These findings confirm and extend the observations of Randall *et al.*[3] A sizable plasma volume decrease was measured in 21 of the 31 patients tested with 150 cc. of 50% glucose with a concomitant rise in hematocrit and classical symptoms of the dumping syndrome. There was excellent correlation between the presence or absence of a history of dumping symptoms and the presence or absence of dumping induced by the test with this amount of hyperosmolar glucose. It is highly significant that in 5 gastrectomized individuals who had no history of clinical dumping and who had no response to the initial testing (150 cc. of 50% glucose), severe dumping manifestations with concomitant plasma volume reductions were produced by the larger test meal (300 cc. of 50% glucose). It is equally significant that large doses of hyperosmolar glucose instilled into the jejunum of normal individuals consistently produced similar florid dumping symptoms and plasma volume changes. On the other hand, oral ingestion of equally large doses of the concentrated glucose solution by normal subjects, in all instances except one, failed to produce dumping symptoms or significant plasma volume changes. Instillation of a large volume of hyperosmolar glucose into the jejunum of dogs consistently produced a reduction in plasma volume, whereas a smaller volume did so only infrequently.

These observations strongly suggest that the dumping syndrome may be consistently induced in any gastrectomized individual by the oral ingestion of a sufficient quantity of hyperosmolar material. It is further suggested that the impressive fluid shifts from the circulating plasma into the intestinal lumen which occur in the dumping syndrome are "physiologic" responses of the jejunum to a sufficient hyperosmolar challenge whether in normal or gastrectomized individuals. By inference the study emphasizes the importance of the pyloric sphincter in preventing jejunal hyperosmolarity under normal conditions. Loss of integrity of the sphincter renders the individual potentially susceptible to the unpleasant features of "dumping".

SUMMARY AND CONCLUSIONS

1. Thirty-one gastrectomized patients were studied following a 150 cc. test meal of hyperosmolar glucose. Twenty-one of these patients showed plasma volume reductions and significant symptoms. Five of those who failed to respond to this meal were retested at a later date with 300 cc. of 50% glucose. All five showed severe symptoms and plasma volume decrease.

2. Ten normal individuals tested by injection of hyperosmolar glucose through an intrajejunal tube exhibited marked symptoms and blood volume changes.

3 None of the controls who took 150 cc. of 50% glucose orally showed any change. One of the 5 normals who drank 300 cc. of 50% glucose had minor symptoms and a significant blood volume reduction.

4. The plasma volume changes to intrajejunal administration of hyperosmolar glucose in the dog followed the same pattern as found in the clinical subjects.

5 These data support the concept that an adequate hyperosmolar concentration in the jejunum will produce the phenomena of "dumping" in both normal and gastrectomized individuals

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ALTERATIONS IN RENAL HEMODYNAMICS IN PATIENTS WITH THE "DUMPING SYNDROME"*

GEORGE C MORRIS, JR, LAZAR JOHN GREENFIELD, AND GEORGE L JORDAN, JR.

An interesting postprandial circulatory disturbance sometimes follows surgical procedures which alter normal gastric drainage This phenomenon has been called the dumping syndrome and is attended with symptoms of weakness, sweating, and tachycardia These symptoms as well as certain objective signs characteristically occur soon after meals of high osmolarity[1] The objective signs in the dumping syndrome include tachycardia and hemoconcentration as well as electrocardiographic and electroencephalographic changes which have been interpreted as a reflection of myocardial and cerebral ischemia[2, 3] It has been postulated that these changes are in direct response to a sudden contraction of blood volume incident to a loss of plasma water within the intestine[4]

However, in this study it was found that symptomatic patients developed an increase in the blood flow to their kidneys during the dumping attack Patients who failed to show objective evidence of the dumping syndrome also failed to show an increase in renal blood flow Furthermore, recent observations by Hinshaw[3] indicate an increased peripheral blood flow during the dumping phenomenon In the light of these findings the simple cause and effect concept between blood volume reduction and the dumping syndrome may need some revision

METHOD

Renal function studies were performed on 16 postgastrectomy patients who presented symptoms suggesting the dumping syndrome Four of these patients failed to show tachycardia or electrocardiographic evidence of the

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dumping syndrome and are considered separately. Renal function was determined using inulin clearance as a measure of glomerular filtration rate (GFR) and low concentration (2 to 1 mg %) of para-aminohippurate to measure renal plasma flow by methods previously described.[5] Cardiac rates were obtained from electrocardiographic tracings and blood pressure by auscultation. Following suitable control determinations each patient was given a liquid meal containing 8 ounces of milk, 6 ounces of 40% glucose and 2 ounces of Ediol. Continuous 10 minute collection periods were then started and continued for the first postprandial hour.

RESULTS

Twelve patients responded to the test meal with tachycardia and electrocardiographic changes compatible with the dumping syndrome. An associated slight elevation in blood pressure was noted in all of these patients. The Billroth I gastrectomy was employed in 4 of the patients and the Billroth II in 8.

During the period from 10 to 20 minutes after the meal the hematocrit was 105% of control. In the successive 10 minute periods of observation the hematocrit was 108, 103, 105 and 101% of control observations. This was statistically significant in all but the last period.

The glomerular filtration rate was 92% of control during the period from 10 to 20 minutes after the meal and 92, 99, 103 and 124% in the successive 10 minute periods of observations. Only the final period was statistically significant.

Renal blood flow was elevated in every postprandial period except in the 10 to 20 minute period. In the subsequent 10 minute periods of observation renal blood flow was elevated to 121, 119, 124 and 146% of the premeal control. These elevations in renal blood flow all approached statistical significance with the exception of the 30 to 40 minute period of observation.

Four patients failed to show electrocardiographic alterations or tachycardia. There was no significant increase in either glomerular filtration rate or renal blood flow. The mean percent of control for both functions tended to be somewhat depressed in the postprandial period. In fact a

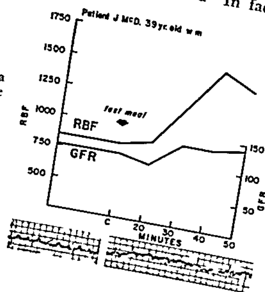


FIG 1. Typical postprandial response of a symptomatic postgastrectomy patient with the dumping syndrome. Though the renal blood flow nearly doubled there was little change in glomerular filtration rate. The electrocardiogram in lead II shows an inversion of the T wave in the postprandial period.

depression in renal blood flow in the 20 to 30 minute period approached statistical significance and was significant in the 40 to 50 minute period

DISCUSSION

From this study it is apparent that there tended to be an increase in renal blood flow during the dumping phenomenon which corresponded with significant electrocardiographic changes Postgastrectomy patients who failed to show evidence of the dumping syndrome also failed to exhibit an increase in renal blood flow In view of these findings previous physiologic concepts of the dumping syndrome may need revision It is difficult to associate a hemodynamic response initiated by a decrease in blood volume which produces increased renal and peripheral blood flow Perhaps more consistent with the findings would be a response triggered by sympathico adrenal stimulation with the release of vasopressor substances

SUMMARY

Renal hemodynamic studies were performed on 12 postgastrectomy patients with manifestations of the dumping syndrome

There was a moderate postprandial elevation in renal blood flow in these patients which approached statistical significance

Similar studies in a series of 4 postgastrectomy patients without manifestations of the dumping syndrome revealed a slight and statistically significant postprandial depression in renal blood flow

Previous hemodynamic concepts of the dumping syndrome based on a response produced by contraction of the blood volume may need further consideration

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STUDIES OF PEPTIC ACTIVITY OF HUMAN GASTRIC JUICE USING TISSUE CULTURE METHODS*

WILLIAM FELLER, AND KAMIL IMAMOGLU

A strictly chemical analysis of the peptic activity of gastric juice has been viewed with suspicion by many investigators It has been felt that the chemical concentration of pepsin alone may not accurately reflect the biological peptic activity of gastric juice The possible existence of naturally occurring pepsin inhibitors or enhancing factors which are operative in living systems has caused investigators to search for biological systems with which to measure peptic activity Perry,[1] *et al*, reported a biological method involving the use of the esophagus of the living cat as a test substrate It utilizes a living anesthetized cat and the results in general have reflected the peptic activity of gastric juice obtained by other methods We have attempted to develop a tissue culture method utilizing human esophageal epithelial cells growing on glass We have employed a strain of human esophageal cells developed here at the University of Minnesota by Dr Jerome Syverton[2] of the Department of Bacteriology It was derived from the normal esophageal mucosa of a two day old infant undergoing surgery for a tracheo-esophageal fistula We have exposed these human cells growing on glass to samples of gastric juice for a period of 3 hours This report describes the methods we have developed and the results which we have obtained using this method to assay samples of human gastric juice

METHOD

Gastric juice was collected from 9 normal fasting subjects who had had no history or symptoms of gastrointestinal disease It was frozen imme diately after collection until it was used Gastric juice from 10 fasting subjects with clinically proven peptic ulcer was collected by an inlying gastric tube attached to a water siphon The collecting bottle was kept on ice during the 8 hour period of aspiration from 11 P M to 7 A M Some of these patients were postgastrectomy individuals, most of them several months, and some were preoperative patients In all cases the diagnosis was confirmed by exploratory laparotomy These samples of gastric juice were also frozen until they were used.

The tissue culture cells were derived from a newborn infant undergoing corrective surgery for a tracheo esophageal fistula A piece of esophageal epithelium from the normal mucosa was used as a source of tissue Sub sequently, cell lines were derived from singly isolated cells These cell lines were carried in a medium of 20% human serum, 0 1% yeast extract, 80% balanced salt solution The cells were transferred serially once a week The procedures used for their derivation and their cultural charac teristics have been reported by Syverton [2] All of the cells used in this study were obtained from Dr Syverton s laboratory As our test system we used a single sheet of these cells growing on a small coverslip in a 15 mm screw-capped test tube The cells were allowed to establish themselves on

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the glass surface for a period of 2 days before being exposed to the gastric juice

The gastric juice samples under study were thawed out and centrifuged to remove any solid sediment All samples of gastric juice were adjusted to a pH of 4 6 with either 0 1 N HCl or 0 1 N NaOH depending upon the original pH of the gastric juice

The cells were exposed to 1 ml of gastric juice for a period of 3 hours at 37°C Following this the cells were returned to nutrient media over night The following morning the cells on the coverslips were fixed and stained The results were read as negative if the cells were remaining on the glass they were read as positive if the cells were off the coverslips indicating cytotoxic damage Each study involved the use of two individual test tubes for each sample of gastric juice under study

Control studies were carried out in exactly the same manner using solutions of purified pepsin of varying concentrations Pepsin determinations were made on all samples by the hemoglobin substrate method of Anson[3] The results of the peptic activity of the gastric juice on the cat esophagus were obtained from Dr Wangensteen s laboratory This method has been previously described by Perry[1] *et al*

RESULTS

The results are summarized in Table 1 and Table 2 They show that in the control group of patients only 2 out of 9 samples of gastric juice had any destructive influence on the cells in tissue culture whereas in the group of patients with diagnosed peptic ulcer 9 out of 10 of the samples

Table 1 Effect of Gastric Juice from Normal Individuals and Patients with Peptic Ulcer on Human Cells in vitro

CATEGORIES	TOTAL NO OF CASES	NO POS	NO NEG	AV VALUE PEPSIN MG /CC
1 Duodenal Ulcer — Postgastrectomy	6	5	1	0 7
2 Duodenal Ulcer — Preoperative	3	3		1 0
3 Gastric Ulcer — Preoperative	1	1		1 5
4 TOTAL OF THE PATIENTS WITH PEPTIC ULCER DISEASE	10	9	1	0 9
5 Control patients — No history or symptoms of gastrointestinal disease	9	2	7	0 6

Table 2 Effect of Solutions of Purified Pepsin at pH 4 6 on Human Cells in vitro

CONCENTRATION OF PEPSIN MG /CC	*Positive* DESTRUCTION OF CELLS	*Negative* NO EFFECT ON CELLS
0 5 mg /cc		x
1 0 mg /cc		x
1 5 mg /cc		x
2 0 mg /cc		x
3 0 mg /cc	x	

of gastric juice exhibited destructive action on the living cells. The correlation with the level of pepsin as measured by the Anson hemoglobin substrate method between the two groups is of questionable significance. While there is a difference between the average or mean value of the two groups equal to 0.3 mg/cc of pepsin, this is probably not significant because the standard deviation within the two groups overlaps the mean value of the other group. In the experimental group which was the only group which was tested, there was no correlation with the results obtained with the cat esophagus method. There was a divergence as often as agreement between the two methods for any given sample. The results with the pepsin solutions at this pH (4.6) show that even with levels of 2 mg/cc *there is no activity against the cells. Destructive activity against the cells* was evident only when the value of pepsin was 3 mg/cc or greater. In none of the active gastric juice samples was a level of pepsin greater than 2 mg/cc recorded. All of these values were determined by the Anson method.

DISCUSSION

The results suggest that the gastric juice of patients suffering from peptic ulcer *is cytotoxic to human cells in tissue culture* at a pH of 4.6 as compared with the gastric juice of normal controls which does not appear to be cytotoxic. Inasmuch as solutions containing purified pepsin in amounts equivalent to the maximum amounts found in active gastric juice are not destructive under these circumstances, it would seem to suggest that the gastric juice of patients suffering from peptic ulcer contains either a specific cytotoxic agent or substances which enhance the activity of acid pepsin against living cells. From the data so far collected it is not possible to tell which of the two possibilities is the more likely. The possible existence of a cytotoxis agent — at least one of an exogenous nature — is not a new postulate. Rosenow[4] in 1913 postulated that a toxin from the streptococci played a role in the etiology of peptic ulcer. He maintained that ulceration of the stomach could be produced regularly in laboratory animals by the intravenous injection of streptococcus isolated from foci of infections in humans. It is interesting in this regard that streptococci isolated from the jejunal ulcers of Mann Williamson dogs were shown to produce acute gastritis, duodenal hemorrhages and ulcers in a high percentage of rabbits.

Because the experiments were run at a pH of 4.6, it might be assumed that the active principle could not be pepsin. In this regard the recent work of Christensen[5] on the optimum pH of peptic hydrolysis is revealing. He directs his attention to the discrepancies in the optimum pH of pepsin which have been obtained by various investigators using different protein substrates. He proposes that the core of this problem is related to the availability of the peptide chains of the substrate molecule and that proteins are rapidly denatured by an acid pH will have a considerable rate of hydrolysis over a broad pH range, i.e., 3.5 to 1.5. He calls attention to the fundamental work of Fruton and Bergman[6] who showed an optimum pH of 4 for the hydrolysis of synthetic peptides. Christensen feels that this result suggests that the low optimum pH for hydrolysis of many proteins may in part be explained by the coiled up structure of their peptide chains.

If this concept is correct, one must be careful in transposing the results of the optimum pH of pepsin on non living proteins to the conditions which exist at the surface of living cells

SUMMARY

1 A biological method of assaying peptic activity of gastric juice using human esophageal cells growing in tissue culture is described

2 The results of studies on various samples of gastric juice using this method reveal that gastric juice from patients with peptic ulcer exhibits a destructive action on living cells at a pH of 4 6 as compared to the innocuous behaviour of gastric juice from normal individuals

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GASTRIC SECRETION AND PEPTIC ULCERATION IN THE DOG WITH PORTAL OBSTRUCTION AND PORTACAVAL ANASTOMOSIS*

THEODORE J DUBUQUE, JR, LEO V MULLIGAN, AND EDWIN C NEVILLE

An increased incidence of peptic ulceration in patients with portal hyper tension has been reported,[1 2] but the occurrence of peptic ulceration after portacaval anastomosis has not been widely recognized Development of upper gastrointestinal ulceration in 15% of 60 patients observed up to 8 years after an end to side portacaval anastomosis stimulated our interest in the relationship of gastric secretion to the portal circulation

Baronofsky and Wangensteen[3] demonstrated that partial interruption of venous return from the stomach in dogs, thus producing a "localized" type of portal hypertension, resulted in a predisposition to histamine induced peptic ulceration To investigate further the background for this predisposition to peptic ulcer, the problem was studied from two stand points First the rate of gastric secretion after complete diversion of portal

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vein blood into the vena cava, as well as after portal vein obstruction, was studied in dogs with Heidenhain gastric pouches Second, in dogs with gastric hypersecretion induced by histamine in beeswax, the potentiating effect on the tendency to peptic ulceration of three operative procedures was studied (1) portal vein ligation, (2) portacaval anastomosis, (3) portal ligation followed in a month by portacaval anastomosis

In the first portion of the experiment, 18 dogs with Heidenhain gastric pouches were studied A stainless steel cannula, similar to the one described by Dragstedt, *et al*,[4] was inserted into the pouch for daily collection of gastric secretion, and the animals were fed a constant diet of canned dog food, milk, and added salt with water *ad lib* throughout the experimental period

After an adequate base line of gastric secretion for each animal had been established, a second procedure was done on each of the animals In 3 of the group this consisted of a control laparotomy alone In 8 of the animals the second operation was a portacaval anastomosis which diverted all of the portal blood into the inferior vena cava by means of a side to-side portacaval anastomosis with complete ligation of the portal vein as close to the liver as possible In the remaining 7 animals, portal congestion was produced by using the method of Popper[5] in which two partially occluding ties of umbilical tape were placed around the portal vein as close to the liver as possible and a few millimeters apart Previous experience with this method invariably resulted in total or near total occlusion of the vein in 10 to 14 days A month or more later a side to-side portacaval anastomosis was done below the site of obstruction in these animals

The performance of a control laparotomy alone had no significant effect on gastric secretion (Fig 1A) The average daily secretion of total acid in these animals was 32 0 mEq prior to the laparotomy and 35 2 mEq following it There was an abrupt and sustained rise following the porta caval anastomosis from an average of 21 0 mEq before to 63 9 mEq following the anastomosis in 8 dogs (Fig 1B) The anastomoses were all widely patent at autopsy Results were similar in the 7 animals that had a partial

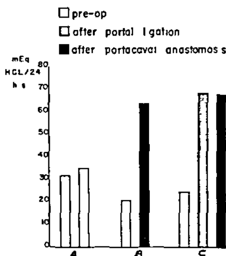


Fig 1

tigated both in gastrectomized dogs and human patients to evaluate the importance of impaired absorption of iron as a factor in the production of the post gastrectomy anemias

Animal Experiments Four normal adult mongrel dogs and 16 gastrectomized dogs that had been operated on 5 months to 5 years previously were used in separate experiments Samples of blood were drawn for determinations of serum iron hemoglobin red blood cell count and hematocrit after which radioactive iron (Fe^{59}) in amounts varying from 50 to 200 microcuries was administered through a gastric tube passed through the mouth Additional blood specimens were then drawn at intervals over a 24 hour period for measurement of their content of radioactive iron Both the normal and gastrectomized dogs were then subjected to intermittent bleeding over a 2 to 3 week period and the Fe^{59} absorption studies were repeated to compare the effect of the bleeding on iron absorption in each group of experimental animals

In the normal dogs (Fig 1a) there was a significant absorption of the radioactive iron within the first 2 hours after its administration During the period following bleeding absorption was increased twofold over that observed during the prehemorrhage period The secondary rise in the

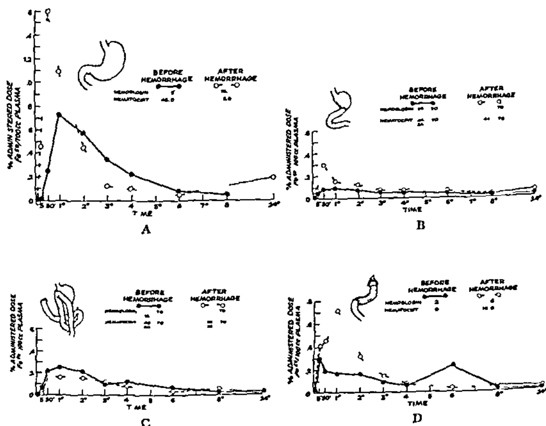


Fig 1 Absorption of radioactive iron before and after bleeding in (a) a normal dog (b) subtotally gastrectomized dogs with gastroduodenostomy (average of 4 dogs) (c) subtotally gastrectomized dogs with gastrojejunostomy (average of 4 dogs) (d) a totally gastrectomized dog with replacement by a segment of colon

plasma isotopic iron levels at 24 hours may be interpreted as an indication of mobilization of labelled iron from storage depots

Subtotally gastrectomized dogs (80 to 85% resection) absorbed much less iron both before and after the hemorrhage than did the normal dogs (Fig 1b and 1c) The animals with gastroduodenostomies absorbed more Fe^{59} after hemorrhage than did those with gastrojejunal continuity (Fig 1c)

All of the totally gastrectomized dogs, regardless of the type of reconstructive procedure, had less absorption of iron before bleeding than did the subtotally gastrectomized dogs (Fig 1d) However, enhanced absorption of iron, which is characteristic of the anemic state in normal animals, was also observed in these totally gastrectomized dogs In fact the absorption of iron in response to bleeding, while not equal to normal, was greater in the totally gastrectomized than in the subtotally gastrectomized dogs (Fig 1b, 1c, and 1d)

Even prior to bleeding the anemia in the totally gastrectomized dogs was more severe than in the subtotally gastrectomized dogs This may be an explanation for the greater absorption of iron in response to bleeding in the totally gastrectomized animals It also emphasizes the fact that a more extensive iron deficiency can be expected to develop after total than after subtotal gastrectomy

Human Patients. Fifteen gastrectomized patients whose operations had been performed 10 days to 8 years previously, and 4 control subjects were included in these studies Samples of blood were drawn for determinations of serum iron, hemoglobin, red blood cells and hematocrit Forty microcuries of radioactive iron were then administered by mouth and blood specimens were drawn at intervals over a 24 hour period for measurement of radioactivity

Totally gastrectomized patients were found to absorb significantly less of the orally administered Fe^{59} than the normal subjects In Figure 2a the results of a radioactive iron study in a patient who 3½ years previously had had a total gastrectomy with replacement by a segment of colon are shown It is apparent that the absorption of iron was far below normal

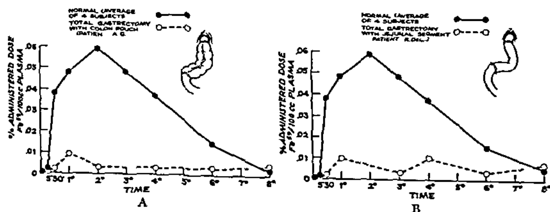


Fig 2 Absorption of radioactive iron in normal subjects and in two totally gastrectomized patients (a) a patient 3½ years after total gastrectomy with replacement by a segment of colon and (b) a patient 3 months after total gastrectomy with replacement by a segment of jejunum

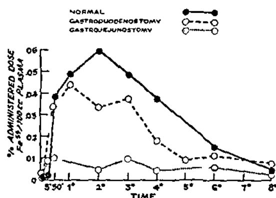


Fig 3 Absorption of radioactive iron in 4 normal subjects and in 2 patients with subtotal gastrectomy—one was a gastrojejunostomy and one with a gastroduodenostomy

In a second patient who had had a total gastrectomy with replacement by a segment of jejunum, studies done 3 months postoperatively also *indicated subnormal absorption of radioactive iron (Fig 2b)*

Figure 3 compares the plasma Fe^{59} levels in 4 normal subjects, and 2 patients with subtotal gastrectomy, 1 with a gastrojejunostomy and the other with a gastroduodenostomy It is evident that the absorption of iron in the subtotally gastrectomized patients was below normal, although not to the extent observed in totally gastrectomized patients As was observed in the animal experiments, the patients in whom gastroduodenal continuity was maintained absorbed significantly greater amounts of Fe^{59} than those with gastrojejunal continuity

SUMMARY

The absorption of iron was studied in gastrectomized dogs and human patients by measurement of the levels of labelled iron in the plasma at intervals after the oral administration of radioactive iron (Fe^{59}) In both dogs and human patients iron absorption after gastrectomy was subnormal The impairment in iron absorption was greatest in totally gastrectomized subjects In both dogs and patients, maintenance of gastroduodenal continuity was associated with more efficient absorption of iron than was the case in subjects in which gastrojejunal continuity was established In normal dogs, iron absorption was markedly enhanced following hemorrhage The absorption of iron in response to hemorrhage after gastrectomy was minimal in the subtotally gastrectomized animals On the other hand, in the totally gastrectomized dogs a significant increase in absorption of iron occurred, although the extent of this increase was not equal to that of normal dogs

THE EFFICACY OF REMOVAL OF PERITONEAL FLUID IN EXPERIMENTAL STRANGULATED INTESTINAL OBSTRUCTION*

William O Barnett and James D Hardy

During recent years there has been a sustained decrease in the mortality rate of patients having intestinal obstruction[2] but most of this improvement has been in the treatment of simple obstruction Progress in the treatment of strangulated intestinal obstruction has not been as impressive

The peritoneal cavity of experimental animals with strangulated intestinal obstruction is the site of collection of large amounts of dark foul fluid When the affected loop of bowel is placed in a plastic bag so that protection from the resulting fluid is afforded death of the animal is indefinitely postponed A rapid downhill course which is fatal within 12 hours or less follows perforation of the plastic bag with escape of the fluid The purpose of this study was to determine (1) the minimum lethal dose of the dark fluid when injected into the peritoneal cavity of normal dogs (2) the length of life following injection of a lethal dose (3) the effect of aspirating the material after a standard period of time and (4)

Table 1 Lethal Effects of Various Doses of Peritoneal Fluid

NUMBER OF DOGS	AMOUNT GIVEN (CC /KG)	NUMBER OF 25 HR SURVIVORS
2	1	2
2	.5	2
3	1 0	3
1	2 0	0
8	3 0	0
2	10 0	0
1	15 0	0
1	18 0	0

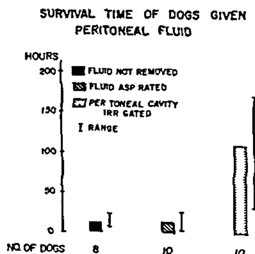


Fig 1 Results in normal dogs following intraperitoneal injection of fluid (3 cc /kg)

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Supported by National Institutes of Health Grant RG-4745

the effect of aspiration of the fluid combined with copious irrigation of the peritoneal cavity after the same length of time It was reported by Burnett[1] that irrigation was beneficial in the treatment of suppurative peritonitis

METHOD

Fifty four mongrel dogs were used for these experiments 6 as donors 20 for determining lethal dose (Table 1), and 28 for evaluating therapeutic measures (Fig 1).

Large adult mongrel dogs were anesthetized with intravenous nembutal Under sterile technique, the terminal ileum was exposed through a lower abdominal midline incision The venous supply to a segment of intestine just proximal to the cecum was divided, following which umbilical tape ties were snugly placed around each extremity of a 10 cm segment All vessels running parallel to the long axis of the bowel at the two sites of obstruction, were divided and ligated (Fig 2) The closed loop of obstructed, strangulated bowel was then returned to the abdominal cavity After closure of the abdominal wound, the animals were placed in their cages At or shortly before death the abdominal cavity was opened and the accumulated fluid was removed It was placed in sterile containers and refrigerated until used

Twenty normal mongrel dogs were given intraperitoneal injections of fluid in amounts ranging from 0 1 to 18 0 cc /kg , and the number of 25 hour survivors was recorded Eight normal animals which received fluid (3 cc /kg) but no treatment were observed for survival time Ten normal animals were given intraperitoneal injections of 3 cc /kg, but the fluid was aspirated from the peritoneal cavity after 5 minutes A third group of 10 normal dogs was given intraperitoneal injections of fluid (3 cc /kg), but in addition to aspiration of the fluid after an interval of 5 minutes the peritoneal cavity was thoroughly irrigated with one liter of isotonic saline or Ringer's solution

RESULTS

The donor animals recovered rapidly from anesthesia, moved about in their cages actively and appeared normal for about 24 hours Thereafter, however, their course was rapidly downhill, and all animals were dead within 5 to 8 hours Depending upon the size of the dog from 200 to 500 cc of dark, foul peritoneal fluid were withdrawn The strangulated segment was found collapsed, although no area of gross perforation was seen The surfaces of the other abdominal viscera were red and inflamed in appearance

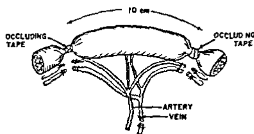


Fig 2 Method of producing closed loop obstruction of strangulated bowel

In the control group the minimal lethal dose was found to be 2 cc /kg (Table 1) It was elected to give the remaining animals 3 cc /kg in order to stay well within the lethal range The 8 animals which received this amount were all dead within 25 hours There appeared to be no correlation between the time of refrigeration of the fluid and the lethal effect The average survival time was 12 2 hours (range 6 to 24 hours)

Ten animals in which the fluid was aspirated from the peritoneal cavity after a 5 minute interval had a mean survival time of 12 7 hours The survival ranged from 3 to 26 hours

Animals which received thorough lavage of the peritoneal cavity with saline solution after aspiration of the fluid lived much longer than those of the preceding groups Four animals recovered completely, and in these chronic survivors the observations were terminated at the end of 1 week Postmortem examination of the 80 95 and 96 hour survivors revealed diffuse peritonitis with multiple abscesses The mean survival time for this group was 108 hours (Fig 1)

SUMMARY AND CONCLUSIONS

The toxic properties of peritoneal fluid resulting from strangulated intestinal obstruction have again been demonstrated Raw fluid, in amounts as small as 2 cc /kg of body weight is lethal when injected into peritoneal cavities of normal dogs One animal may produce enough fluid to kill 7 dogs of similar size When a lethal dose of fluid (3 cc /kg) is placed in the peritoneal cavity of a normal animal simple aspiration after a 5 minute interval did not improve the survival time over the control group There was a significant prolongation of life if the peritoneal cavity was thoroughly irrigated with saline after aspiration of the dark fluid from the donor animal

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Liver and Biliary Tract

STUDIES ON LIPID METABOLISM IN DOGS WITH ALTERED BILIARY PHYSIOLOGY*

STEVEN G ECONOMOU, BEVERLY J TEWS, C BRUCE TAYLOR, AND G E COX

The biochemistry and physiology of bile appear to be intimately involved in the pathogenesis of both cholelithiasis and atherosclerosis This truth is a reflection of the central position of the liver (and especially its secretion – the bile) in the metabolism of cholesterol Synthesis of cholesterol by the liver has been frequently demonstrated and has been generally assumed to occur at a very rapid rate and to be of primary importance in the production and maintenance of hypercholesterolemia in the human Earlier work in this laboratory indicated a very low rate of synthesis of cholesterol by human liver tissue [1] Extensions of this work led to an effort to isolate some of the individual factors concerned in the regulation of cholesterol synthesis in liver tissue Several factors were already known Dietary cholesterol markedly suppresses hepatic cholesterol synthesis [2] Starvation has a similar suppressive effect [3] Biliary stasis has been variously reported to increase the synthetic rate[4] and to have no effect on the synthetic rate of cholesterol in the liver of rats [5]

These experiments were designed to measure changes in the concentration of cholesterol in blood and liver and changes in the synthesis of cholesterol by liver tissue, in dogs subjected to various alterations of biliary physiology The alterations so far studied include biliary stasis, biliary fistulae and dietary administration of bile and bile acids to normal and fistulous dogs

METHOD

Two sets of experiments were done using adult mongrel dogs on a low fat low cholesterol diet In the first experiment, 7 dogs had control liver biopsies and complete double ligation and severance of the common duct under nembutal anesthesia After 2 to 4 weeks a repeat surgical liver biopsy was taken In the second set of experiments, 8 dogs under general anesthesia had control liver biopsies, complete ligation of the common duct and a right nephrectomy followed by anastomosis of the fundus of the gall bladder to the right renal pelvis This permitted a free flow of bile out through the urinary system These animals had repeat liver biopsies under general anesthesia at 3 weeks, some at 6 weeks and even as long as 13 weeks after the original fistula operation

Immediately after excision of the liver biopsy, the tissue was sliced into

*From the Department of Surgery University of Illinois College of Medicine and the Presbyterian Hospital Supported by funds from the American Illinois and Chicago Heart Associations, The Life Insurance Medical Research Fund and the Otho S A Sprague Memorial Institute

thin sections and incubated with C^{14} labelled sodium acetate The choles terol was then extracted, precipitated with digitonin and the radio activity determined with an end window Geiger counter Aliquots of the original samples were analyzed for total cholesterol by the Schoenheimer Sperry method The radioactivity of the newly formed cholesterol was calculated and expressed as milligrams of cholesterol synthesized per 100 grams of liver tissue per hour (mg /100 gm /hr)

For additional bile feeding experiments, fresh dog bladder bile was obtained from animals at the time of sacrifice Human bladder bile was obtained in the course of autopsies The bile was stored frozen or at 4°C It was administered via stomach tube after mixing with a small quantity of food and water

Whenever possible, each animal was used first as a control, so that its later experimental data could be compared directly with its own control data Because of some deficiencies in individual controls, however, measure ments were also made on numerous other control dogs, and their average values used to confirm or supplement the control values of the experimental animals

RESULTS

The serum total cholesterol in 19 control dogs ranged from 109 to 221 mg %, with an average of 153 mg % The liver total cholesterol in 30 control dogs ranged from 186 to 289 mg % with an average of 235 mg % The hepatic cholesterol synthesis rates in 21 control dogs ranged from 0 2 to 1 2 (average, 0 65) mg /100 gm /hr

Choledochal ligation resulted in jaundice and alcoholic stools In the presence of total bile stasis, following a second surgical liver biopsy, the raw liver surfaces oozed bile and the animals developed bile peritonitis Consequently, the animals were unsuitable for repeat liver biopsies at a later date After 2 to 4 weeks of biliary stasis, the serum total cholesterol

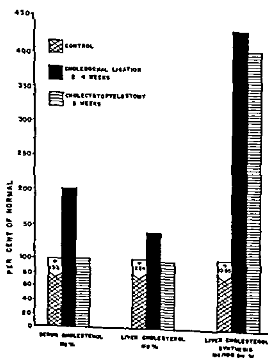


Fig 1 Effects of biliary stasis and biliary fistula upon some aspects of cholesterol metabolism in dogs In the bars for the control animals the numbers below the arrows represent the absolute value for the average of the controls Thus, 153 means that 153 mg % was the average serum total cholesterol in control dogs The average serum cholesterol in bile duct ligated dogs was 202% of 153 or 310 mg % In the third set of bars (for liver cholesterol syn thesis) the control animals had an average rate of 0.55 mg /100 gm /hr

levels ranged from 210 to 408 mg %, with an average of 310 mg % The liver total cholesterol ranged from 229 to 373 mg %, with an average of 310 mg % The hepatic cholesterol synthesis rates ranged from 1 1 to 3 7, with an average of 2 2 mg /100 gm /hr Figure 1 gives a graphic summary of the data presented in this report

In the second set of experiments, the cholecystopyelostomy animals were not accepted unless they had alcoholic stools and no jaundice Blood and liver tissue were also checked for evidence of bile stasis Of the acceptable animals, some ate poorly and to avoid this variable, the animals were tube fed daily a quantity of food adequate to maintain their weight The repeat surgical liver biopsy and blood sample were taken together, generally 3 weeks after the initial operation At this time the serum total cholesterol ranged from 123 to 216 mg %, with an average of 153 mg % The liver total cholesterol ranged from 180 to 284 mg %, with an average of 219 mg % The hepatic cholesterol synthesis rates ranged from 1 2 to 3 8, with an average of 2 4 In several animals that had repeat biopsies at 6 and 13 weeks, the hepatic cholesterol synthesis rates remained essentially at the 3 weeks level

In preliminary experiments the oral administration of crystalline bile acids have yielded variable and inconclusive results Dog bladder bile fed to normal dogs produced no suppression of hepatic cholesterol synthesis Human bladder bile fed to normal dogs suppressed synthesis rates to one half to one fourth of their respective initial control rates Dog bladder bile fed to bile fistula dogs reverted the cholesterol synthetic activity of the liver part way back to normal When the last dose was administered 24 hours before the liver biopsy, dog bladder bile had no effect upon the synthesis rates in bile fistula dogs, the partial suppression was obtained by feeding several small bile meals 3 to 10 hours before the liver biopsy Some dogs tolerated 2 meals of 50 to 75 ml dog bladder bile 3 to 5 hours apart, other dogs had diarrhea with loss of some of the administered bile

DISCUSSION

The fourfold elevation of hepatic cholesterol synthesis rates in dogs with acute (generally 2 weeks) biliary stasis, confirms the fourfold elevation previously observed in the liver of rats with acute (1 to 2 days) biliary stasis [4] The fourfold elevation of hepatic cholesterol synthesis rates in dogs with a biliary fistula compares very well with the fourfold increase in output of bile acids observed in other dogs with biliary fistulae [6] These two opposite biliary conditions thus exert a similar and equal influence on hepatic cholesterol synthesis Possibly they act in opposite ways or possibly both act in the same way A possible common denominator might be their block of the enterohepatic circulation of bile, with a resulting block in intestinal absorption of cholesterol Even in animals ingesting no cholesterol, biliary drainage and desquamation of intestinal epithelium probably provides some cholesterol for absorption or reabsorption This concept appears to derive some support from the demonstration of increased hepatic cholesterol synthesis rates in rats with a thoracic duct canula [7]

It is not surprising that a biliary fistula accelerates hepatic cholesterol synthesis Various experimenters through the past several decades have

demonstrated increased production of bile acids in biliary fistula animals Recent demonstrations of the origin of bile acids chiefly from cholesterol, appear to require accelerated hepatic cholesterol synthesis as part of the process of accelerating bile acid output.

The relation of the time of feeding to the suppression of hepatic cholesterol synthesis by oral administration of dog bladder bile to bile fistula dogs, appears understandable in the light of the concept of a bile acid pool[8] This pool apparently consists of bile acids in transit and in the tissues of the gastrointestinal tract, blood, and liver. The pool is rapidly depleted in a free flowing bile fistula and administered bile likewise freely flows out, with only a transient elevation of the bile acid pool toward a normal level Timing the feeding and the surgery to make the time of biopsy and incubation coincide with the time of this transient peak, probably is the explanation of our findings.

The suppression of hepatic cholesterol synthesis in normal dogs fed human bladder bile, with no suppression by dog bladder bile, probably is simply a reflection of the much greater amount of cholesterol in human bile

SUMMARY

1 The hepatic cholesterol synthesis rate is accelerated fourfold in dogs with biliary stasis for 2 to 4 weeks

2 The hepatic cholesterol synthesis rate is accelerated fourfold in dogs with an internal biliary fistula for 3 to 6 weeks

3 The hepatic cholesterol synthesis rate of normal intact dogs appears to be suppressed by cholesterol rich bile much the same as by other cholesterol rich diets

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STUDIES ON THE PRODUCTION OF BILIARY CONCREMENTS WITH 3 BETA CHOLESTANOL IN LABORATORY ANIMALS*

EUGENE G CAIRA, STANLEY C SKORYNA, A C RITCHIE, AND D R WEBSTER

The incidental observation by Cook[1] during experiments in atherogenesis that 3 beta cholestanol induced gallstone formation, and further investigation by Mosbach[2] suggested to us that this simple and near physiological method of producing calculi afforded a means of studying the formation of one type of calculus from its inception to any desired age There are only a few instances recorded in the literature where the exact age of human calculi was known (Mentzer[4]) The prevailing intense interest in serum cholesterol values in humans, and the administration of drugs like 3 beta cholestanol to lower cholesterol levels also prompted the administration of this drug to rabbits, guinea pigs and rats to ascertain if there was any variation in species response In an attempt to ascertain whether or not the gallbladder was necessary for the formation of calculi, rabbits were *cholecystectomized and then fed the drug* Routine bacteriological studies were carried out to eliminate any possibility that the pathology produced was due to bacterial agents

METHOD

Experiment 1: Twenty New Zealand rabbits of both sexes, weighing approximately 3 kg, were fed 0 5 gm of 3 beta cholestanol daily for periods up to 21 days Water was given *ad libitum* The Bevans'[3] method of preparing the pellets, by dissolving the drug in absolute ethyl ether and pouring it over a weighed quantity of pellets, was used The rabbits were thus given 40 gm of pellets containing a desired dose of drug and 60 gm of Purina pellets were given when this was consumed After 5 days on the diet, the animals were sacrificed in pairs at 4 day intervals After 21 days on the diet, two pairs of rabbits were given untreated pellets only for further periods of 10 to 20 days, respectively

Experiment 2: Fourteen rabbits weighing approximately 4 kg were cholecystectomized using intravenous Nembutal and the usual surgical technique One week later they were started on 0 5 gm of 3 beta cholestanol daily for 21 days and then sacrificed

Experiment 3: Twenty guinea pigs of both sexes, weighing approximately 600 gm were fed 0 25 gm of 3 beta cholestanol daily and sacrificed as in Experiment 1

Experiment 4: Twenty rats of both sexes of the Royal Victoria Hospital strain, weighing approximately 250 gm were fed 0 125 gm of the drug daily and treated as in Experiment 1

Blood and bile specimens were taken at autopsy for cholesterol and pH estimations Samples of concrements gallbladder, bile and common bile duct were selected for bacteriological culture Sections were taken for H & E, trichrome staining and histopathological studies

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RESULTS

Experiment 1. In all cases after the 9th day, cholelithiasis developed In 3 rabbits common bile duct concrements were observed The quantity of concrements varied directly with the length of time on the diet After 21 days the gallbladder in one rabbit was packed solid so that a soft whitish green cast was formed The rabbits thrived on the diet and progressively gained weight The concrements found in the earlier sacrificed animals were of gelatinous consistency and an opaque whitish-green color (See Fig 1) At 21 days a mixture of these soft concrements with light green crystalline calculi was found (See Fig 2) The presence of free particles of dark pigmented material was noted admixed with the concrements (See Fig 3) The calculi appeared to have increased in size by a method of aggregation comparable to the coalescence of butter fat globules in a churn Their average size was not greater than 1 25 mm Some crystalline forms were stained with blood pigment (See Fig 2) The quantity of bile was markedly limited by the number of calculi or concrements present and where present was thick and gelatinous

The histopathological findings were similar to those of Bevans[3] In one section 4 plus edema with round cell infiltration extended throughout the gallbladder wall and serosal fibrosis was present Common bile duct changes were similar, and one rabbit showed a grossly hemorrhagic gall bladder at autopsy with marked inflammatory response microscopically, the crystalline calculi were blood stained in this case The pathological changes in the gallbladder and ducts became more pronounced the longer the period the animals were fed 3 beta-cholestanol The biochemical analyses showed a significant reduction of bile and blood cholesterol levels

Experiment 2: Two rabbits had common bile ducts almost packed solid with concrements, and three others showed fewer concrements The ducts exhibited a marked inflammatory response These animals also thrived well and no signs of jaundice were noted The blood cholesterol levels in these animals were moderately depressed

Experiment 3: Two guinea pigs developed gallbladder calculi (Fig 4)



Fig 1 Photomicrograph of gelatinous aggregates Rabbit fed 3 beta cholestanol 13 days (x 140)



Fig 2 Photomicrograph of crystalline calculi (green and blood stained) Rabbit fed 3 beta-cholesterol 21 days. (x 140)



Fig 3 Photomicrograph of soft gel deposit forms plus pigment particles Rabbit fed 3 beta cholestanol, 9 days. (x 140)



Fig 4 Photomicrograph of soft and crystalline orange colored deposits in guinea pig fed 3 beta cholestanol 21 days (x 140)

with mild cholecystitis present The animals thrived well on the diet and no significant reduction in blood and bile cholesterol levels was noted The orange colored calculi were admixed with gelatinous forms and aggregations of the hard and soft material were noted

Experiment 4: No concrements were found in the common bile ducts and the sections examined microscopically showed no pathological changes The biochemical analyses yielded similar findings to those of the controls

The bacteriological studies in Experiments 1, 2 and 3 were negative and 4 cultures of bile from rats yielded the following organisms *c coli, p mirabilis, s viridans, s thermophilus, gaffkya tetragena, b sublilis* in various combinations These were considered contaminants, but a larger study to determine the normal flora of the biliary tract in rats is needed

Chemical and spectrophotometric analyses of the calculi and concrements revealed that they consisted mainly of bile acids and had a low cholesterol content (Mosbach [2])

No significant changes in the pH of the bile samples were noted in the animals with concretions present

COMMENTS

There is a definite species difference in the metabolism of 3 beta cholestanol in the animals studied, resulting in variations in the pathology and incidence of biliary calculus formation In rabbits, the gallbladder plays no part in the production of calculi induced by feeding 3 beta cholestanol It is possible that a larger percentage of the cholecystectomized rabbits formed minute 'gel concretions in the common bile duct which were passed into the duodenum before autopsy was carried out The fact that the rats showed no concrements in the common bile duct may be due to a similar mechanism or some species difference in the reciprocal duct and sphincter activity mechanism of animals which normally have no gall bladder The effect, if any, of the bacteria isolated in Experiment 4 on the bile in the common ducts of rats has not yet been ascertained A liver dysfunction or overfunction appears to be the primary factor in the patho genesis of the biliary changes observed It seems probable that dietary variations can upset the liver homeostatic mechanism and cause secretion of abnormal toxic bile or disturb the bile acid cholesterol critical ratio The marked inflammatory reaction of the gallbladder in response to the presence of excess bile acids has been confirmed (Liaw [5])

SUMMARY

The production of calculi by the oral administration of 3 beta cholestanol (dihydrocholesterol) to rabbits and guinea pigs has been shown No pathology was detected in rats fed the drug Cholecystectomized rabbits developed choledocholithiasis when fed 3 beta cholestanol A definite variation in species response has been demonstrated in the animals used

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FORMATION OF CALCULI FOLLOWING CHOLECYSTECTOMY ATTENDING PARTIAL OCCLUSIONS OF THE COMMON BILE DUCT*

Kamil Imamoglu, Earl G Yonehiro, John F Perry, Jr., and Owen H Wangensteen

In the past[1,2,3,4,5,6] extensive experimental studies have been carried out to elucidate the etiology of concretions in the biliary system and infection[1,2,3,4] metabolic alterations[7] and stasis as causative factors have been stressed[1,2,3,4,5,6,7,8] It has been observed in this clinic[9] that patients with cholelithiasis frequently have an abnormal degree of narrowing of the terminal common duct at the papilla A method of experimentally producing gallstones by incomplete stricture of the distal common duct simulating this condition has been described[9] Insofar as persistent or recurrent common bile duct stones following cholecystectomy has been shown to be associated in some patients with strictures of the papilla of Vater or stenosis of the sphincter of Oddi it has been conjectured that this partial biliary outflow obstruction may be the responsible factor in stone formation

The study reported herein appears to indicate that under the conditions of the experiment narrowing of the terminal common duct may indeed result in the appearance of stones in the common duct, hepatic ducts as well as in the major intrahepatic biliary system This observation confirms

*From the Department of Surgery University of Minnesota Medical School Minneapolis Minnesota Supported in part by U S P H Grant #RG1028(c) Austen S and Anne R Cargill Jay and Rose Phillips Funds for Surgical Research

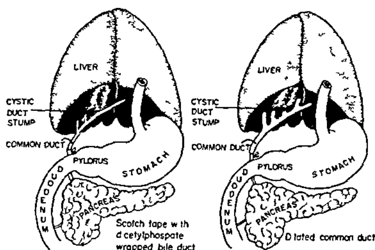


Fig 1 Experimental technique utilized to produce stricture of the distal common bile duct with fibrosing agent

the impression that narrowing at the ampulla may be responsible for persistent or recurrent choledocholithiasis

METHOD

Two animal species, the dog and rabbit, were used for these experiments Under pentobarbital anesthesia and utilizing aseptic technique, the gall bladder was removed in the routine retrograde manner, with reperitoneal ization of the gallbladder bed upon completion of the cholecystectomy After exposure of the common bile duct at its site of entrance into the duodenum, a small strip of cellophane sealing tape, dusted lightly with dicetyl sodium phosphate, was sutured very loosely around the terminal common duct as it penetrated the duodenal wall (See Fig 1) This substance (dicetyl phosphate) is well known for its property to stimulate fibrosis After instillation of one-half million units of aqueous penicillin into the peritoneal cavity, the abdomen was closed with interrupted silk sutures

The animals were fed regular laboratory rations and received no anti biotics or special postoperative care All animals that died were autopsied while the others were sacrificed at varying intervals of time Aerobic and anaerobic bacterial cultures of bile and tissue were obtained from the common duct, hilus, and liver of a number of the animals at the time of sacrifice Bilirubin levels were obtained also at periodic intervals during the study At autopsy or sacrifice the biliary system and liver were carefully examined Particular attention was given to the degree of stenosis at the papilla, the condition of the ducts, and the nature of bile and stones found

RESULTS

All 8 rabbits (100%) utilized in this experiment developed stones in the common bile duct and hepatic ducts In addition, stones were present in the intrahepatic ducts in every case Concretions were also present in 3 of the 6 dogs (50%) used in this study The results of the experiment are summarized in Table 1 The distal common duct was incompletely occluded in every case, allowing the free flow of bile or the passage of

a 1 mm probe Proximal to the stenosis, the duct was only moderately dilated, no evidence of jaundice could be demonstrated clinically, although one animal showed slight elevation of bilirubin (Table 1) In dogs (Table 2), common bile duct stones occurred as early as 5 weeks following cholecystectomy and the application of the stenosing agent about the duct, while stones in rabbits developed as early as 12 weeks after the operation
The chemical composition of the stones obtained from rabbits were

Table 1 Stones in Biliary Tract Following Cholecystectomy and Experimental Stricture of Terminal Common Bile Duct

SPECIES	LENGTH OF SURVIVAL TO DEATH OR SACRIFICE (WEEKS)	STONES	TYPE OF STONES
Dogs #			
1	43	Present	Cholestrol Pigment
2	25	Present	Calcium Cholestrol Pigment
3	8	0	—
4	5½	0	—
5	5	Present	Pigment
6	3	0	—
Rabbits #			
1	12	Present	Cholestrol
2	13	Present	Cholestrol Pigment
3	17	Present	Cholestrol Pigment
4	18	Present	Cholestrol Pigment
5	21	Present	Cholestrol Pigment
6	21	Present	Cholestrol Pigment
7	28	Present	Cholestrol Pigment
8	30	Present	Cholestrol Pigment

Table 2 Serum Bilirubin Values in Rabbits with Experimentally Produced Biliary Stones

ANIMAL NUMBER	NUMBER OF WEEKS POSTOPERATIVE	BILIRUBIN MG PER CENT	
		ONE MINUTE	TOTAL
1	11	01	05
2	13	05	9
3	17	1	9
4	18	.3	1 8
5	21	06	1.0
6	20	1	.2
7	28	05	1 0
8	30	05	.9

cholesterol and pigments and pure cholestrol in composition, bilirubin or calcium cholesterol bilirubin stones were seen in the dogs

Cultures of bile, liver, hilus, and common bile duct were taken from 6 rabbits Four of these were sterile Escherichia coli was obtained from the biliary tree of 2 rabbits X ray studies of the biliary tract using oral Telepaque (Iodopanoic acid, Wintrop laboratories) and intravenous Cholografin (Iodipamide methylglucamine, E R Squibb & Sons) have not been helpful in demonstrating concretions in these animals at any time prior to death

DISCUSSION

The incidence of stones in the common duct in cases of calculous cholecystitis in man has been variously reported as high as 22%[10] We have observed that in 58% of patients with cholelithiasis narrowing of the duct at the ampulla was present such that a 3 mm probe could not be passed easily into the duodenum

Cholecystectomy and the experimental production of biliary stasis by partial obstruction to the terminal common bile duct in the manner described herein will result in the development of stones in the common bile duct, the hepatic ducts, as well as in the larger intrahepatic ducts As emphasized earlier,[9 11 12] it would appear that narrowing or fibrosis of the sphincter of Oddi might be an important factor in the production or recurrence of common duct stones in man Our observations appear to support this thesis Furthermore, presence of the gallbladder is not essential for the production of stones Rufanov[13] has amply discussed the role of liver stones in cases of choledocholithiasis In addition, our studies indicate that an infectious component is not an essential factor for stone development in the biliary tree

Experiments[4 5] using pancreatic juice activated by bile suggest that a stenosing choledochitis may be produced in this manner Additional observations in this clinic have demonstrated the great sensitivity of bile duct epithelium to the action of gastric juice as assessed by perfusion studies Whether it is an acid peptic factor, periodic spasm of the sphincter as suggested by Cattell,[11] or a combination of factors, some of which remain unknown, that initiate anatomic narrowing of the sphincter of Oddi or of the papilla, leading eventually to stone formation needs further elucidation Some such mechanisms are currently under study in this laboratory

SUMMARY

Stones in the common hepatic, and intrahepatic duct have been observed following cholecystectomy attending the experimental production of partial obstruction of the terminal common bile duct Stasis is the important agent in the causations of these stones Infection is not essential to the experimental production of cholelithiasis A stenosed or narrow papilla of Vater or sphincter of Oddi must be corrected to avoid recurrent choledocholithiasis in man

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DEMONSTRATION OF IMPAIRED BLOOD FLOW THROUGH THE LIVER FOLLOWING CIRCULATORY STASIS*

M DON TURNER, WILLIAM O BARNETT, GEORGE W TRUETT, AND JAMES C GRIFFIN, JR

Prolonged hemostasis to any portion of the body or to the whole body results in irreversible alterations Many of these changes, which are still little understood are incompatible with the life of the tissues even though the blood flow be restored The following study demonstrates the diminished functional vascularity of liver and muscle tissue after various periods of circulatory stasis The results of this work firmly support Crowell's hypothesis of irreversible shock [1]

METHOD

The various periods of circulatory stasis to the liver were based upon the studies of Raffucci [2] who demonstrated a 100% mortality following clamping of the afferent blood supply to the liver for one hour and a 75% mortality after a 30 minute period of stasis

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A collimated scintillation scanner was positioned over the exposed liver of dogs Sodium 24 was injected into the liver tissue directly under the scintillator Continuous recordings of the Na^{24} clearance rates were obtained from the liver tissue of the dogs The time required for half of the injected radiosodium to disappear from the liver was calculated This value is the sodium 24 clearance half time from liver tissue The radiosodium clearance was determined before occlusion of the hepatic artery and portal vein *Clamps were placed about the vessels, then removed* after varying periods of time and the clearance rates again determined The following hemostasis periods were employed

GROUP NO	NO OF ANIMALS	STASIS PERIOD (MINUTES)
1	7	10
2	7	15
3	7	30
4	7	60

In a group of 3 dogs, radiosodium clearance curves were obtained from the hind limb muscle before and after occlusion of the aorta below the kidneys for one hour

RESULTS

From these experiments it appears, on the basis of radiosodium half time values, that after occlusion of the blood flow to the liver the degree of impairment of the circulation to liver tissue depends upon the stasis time After occlusion for 10 minutes the radiosodium clearance rates from liver tissue is only slightly retarded (Fig 1)

Occlusion of the blood supply to the liver for 15 minutes was followed by clearance rates about 4 times slower than control rates Occlusion for 30 minutes was followed by rates approximately 6½ times slower than normal The mean radiosodium half times in dogs subjected to one hour occlusion were found to be almost 12 times slower than control values Two animals demonstrated complete inability to clear sodium 24 after one hour of circulatory arrest to the liver In 28 normal dogs the radiosodium clearance half time in liver was 1 23 min

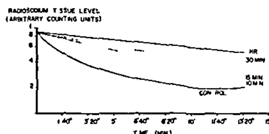


Fig 1 Radiosodium clearance curves from the liver of dogs before and following periods of circulatory stasis

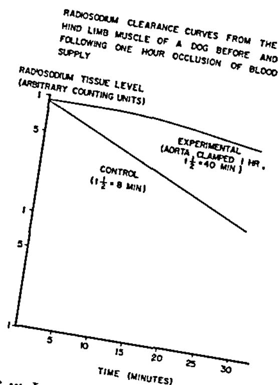


Fig 2 Radiosodium clearance curves from the hind limb muscle of a dog before and following one hour occlusion of blood supply

Table 1 Sodium 24 Half Time in Liver and Muscle

TISSUE	NORMAL ½ T (MIN) (RANGE)	(MEAN)	STASIS PERIOD (MINUTES)	AFTER STASIS ½ T (MIN) (RANGE)	(MEAN)
Liver	0 67 to 1 67	1.23	10	1.33 3 00	1 88
Liver			15	1 72 8.50	4 87
Liver			30	3 80 10.33	6 63
Liver			60	6 67 18 17 (No clearance in two dogs)	11.95
Muscle	8 0 15 0	11 00	60	25 00-40 00	30 67

Figure 2 demonstrates the diminished sodium 24 clearance rates in muscle after similar one hour periods of arrested circulation

Table 1 summarizes the sodium 24 half times in normal liver and muscle and in these tissues after circulatory stasis

DISCUSSION

It is known that following circulatory arrest to an organ the hydrogen ion activity of that tissue increases rapidly, overcomes the buffer systems in the immediate vicinity, and causes a rapid decline in pH [2] A recent hypothesis[1] set forth to explain, in part, the sequelae of irreversible shock states that the diminishing pH is a direct cause of the formation of miliary blood clots These minute clots obliterate a large number of capillaries Even though the blood supply be restored to the tissue the capillaries remain nonfunctional, thus preventing adequate perfusion of the tissue. The architecture of the capillaries and of the tissues clearly betrays diffusion as the chief mechanism of transport. As the number of functional capillaries decrease the distance over which a material must diffuse to and from other

patent capillaries increases and the time required for materials to traverse these distances multiplies rapidly

We are of the opinion that the clearance of a highly diffusible ion such as sodium measures to some extent the functional vascularity of tissue or in other words the efficiency with which blood perfuses a tissue The extent to which the radiosodium tissue clearance rates depend on the actual *blood flow* to organs has not yet been elucidated

CONCLUSIONS

1 Even short periods of circulatory stasis to an organ impair the efficiency with which the blood can perfuse the tissue

2 As the period of occlusion becomes greater the degree of circulatory impairment increases proportionately until a condition of irreversibility is attained which cannot be overcome by a restored blood supply

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THE EFFECT OF HEPATECTOMY ON THE PROTEIN COMPONENTS OF PLASMA*

JAMES G STEPHENS RICHARD A BAHN J FOPEANO AND WORTHINGTON G SCHENK JR

The association of disorders of the liver with the diminution of the plasma proteins has been well known in the clinical studies of the blood in patients with hepatic disease Previous investigators have shown that the liver has a definite role in the regulation of the protein content of the plasma However indefinite or only slight changes in the plasma proteins were reported and most of the experimental hepatectomy series had poor survival times Thus we felt further investigation into these changes was warranted It was not clear whether the disturbance in the normal pattern was due to lack of formation of proteins abnormal utilization proteolysis or indirect loss from the circulating plasma into the extravascular tissue spaces To aid in answering these questions we have observed the effects that total removal of the liver might have on the plasma proteins With removal of the liver we have removed the source of formation of the plasma proteins and thus we could study the specific effect that the liverless animal has on the plasma proteins

*From the Department of Surgery University of Buffalo Edward J Meyer Memorial Hospital Buffalo New York Supported in part by U S P H Grant #H 613(C6)

METHOD

The livers of healthy adult dogs were removed by the two stage method of Markowitz [1] The first stage consisted of partial occlusion of the portal vein and inferior vena cava with complete division of the gastrohepatic ligament The second stage was carried out 6 weeks later and the entire liver was removed Following hepatectomy the animals were maintained by intravenous infusion of 7½% glucose in ⅓ normal saline given at a constant rate of ¼ gm of glucose per kilogram per hour Urine and blood samples were taken at 4 hour intervals Lymph determinations were made by placing a catheter in the thoracic duct

RESULTS

A total of 16 animals was studied in this series with a survival time of 16 to 36 hours following complete removal of the liver The changes which occur in the plasma proteins within 12 hours after hepatectomy are relatively small however the downward trend is definitely established The fall in plasma proteins is illustrated graphically in Figure 1 which indicates the concentration of the plasma proteins is approximately 15% below normal at 12 hours following hepatectomy In the 12 to 28 hour period the fall in proteins continues and at 24 hours following hepatectomy, all the plasma protein values are approximately 50% of their preoperative level *This decrease in plasma proteins takes place despite a constant* hematocrit or in some cases slight hemoconcentration This decrease in plasma proteins was also demonstrated by the electrophoretic technique as shown in Figure 2 The relative mobility and contour of the various protein peaks show a gradual decline on the electrophoretic graphs The nonprotein nitrogen blood levels were studied and the results were comparable There was a constant decrease in the nonprotein nitrogen from a preoperative value of 33 9 mg % to 17 6 mg % at the 24 hour posthepatectomy period All animals had good urinary output during their postoperative course which indicated good urinary function The total nitrogen excreted in the urine during the first 12 hours following hepatectomy was within normal limits averaging from 1 3 to 2 1 gm However in the 12 to 28 hour period the total nitrogen excretion was markedly decreased and was far below normal urinary excretion The urinary determinations of ammonia nitrogen urea nitrogen creatinine nitrogen and protein nitrogen followed similar patterns The total protein nitrogen excreted was of insignificant amount Thoracic duct flow and the protein concentration of thoracic duct lymph were studied in 3 dogs The volume of thoracic duct flow was greatly increased in the hepatectomized animal and the protein concentration of thoracic duct lymph fell in the same fashion as the protein concentration of the plasma There was also an increased mixing rate between plasma and lymph after I^{131} labelled albumin was injected into the circulating blood of the hepatectomized animal

DISCUSSION

The direct effect of hepatectomy is the removal of the supply of the plasma components because of the lack of hepatic synthesis It is not known to what extent extrahepatic synthesis supplies the plasma proteins

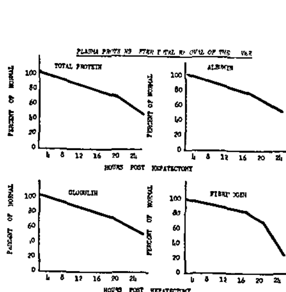


Fig 1

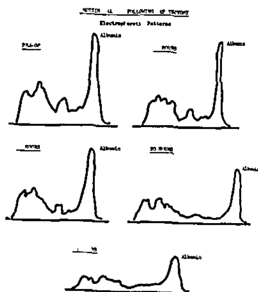


Fig 2

it is thought however that the extrahepatic sources yield very little The normal utilization of approximately 10% of the circulating plasma proteins in a 24 hour period is not sufficient to account for the observed reduction following hepatectomy A plausible explanation of the changes observed in the plasma would be that the depletion is the result of increased extra hepatic utilization and absent hepatic synthesis However if this were true there should be some evidence of protein breakdown products in the plasma or evidence of the excretion of these products in the urine The nonprotein nitrogen of the plasma decreases from a preoperative level of 33 9 mg % to a low of 17 6 mg % despite an increase in the serum amino acid levels Additional evidence against this theory are the urinary findings which show normal urinary excretion of total nitrogen during the first 12 hours following hepatectomy and a marked decrease in the total nitrogen excretion in the 12 to 28 hour period when the protein loss is greatest The protein nitrogen excreted in the nitrogen is of insignificant amount and could account for only a minute fraction of the total protein loss These urinary determinations are done while the animal is maintaining a normal urinary output and thus we assume has adequate kidney function We may then conclude that the proteins are not undergoing proteolysis due to increased plasma or proteolytic activity nor is the decrease due to increased utilization of circulating plasma proteins from some abnormality in protein metabolism The other alternative is indirect loss of plasma proteins that is leakage of the components into the extravascular spaces due to increased capillary permeability This theory may be substantiated by the fact that the posthepatectomy animal has a relatively constant hematocrit or may even show some hemoconcentration despite the generalized bleeding tendency which is apparent late in the posthepatectomy period Indirect evidence of increased capillary permeability and intercellular edema is the greatly increased thoracic duct flow in the hepatectomized animal Other evidence of increased capillary permeability is the greatly accelerated mixing rate or rate of equilibration between plasma and

lymph following injection of I^{131} labelled albumin into the circulating blood volume Thus the mechanical factor of plasma loss may account for the constant changes observed with such an effect being produced by the loss of the liver itself causing an increase in capillary permeability

CONCLUSIONS

1 Quantitative changes have been demonstrated in the plasma proteins in 16 dogs following hepatectomy

2 The decrease begins immediately but the changes become more marked in the 16 to 24 hour period postoperatively

3 At the 24 hour period posthepatectomy the plasma proteins are approximately 50% of their preoperative level

4 These changes have been demonstrated by electrophoretic patterns

5 There is a constant decrease in the blood nonprotein nitrogen and the total nitrogen excretion in the urine is within normal limits during the first 12 hours postoperatively and is less than normal in the 12 to 24 hour period

6 We assume this decrease in plasma proteins is not due to proteolysis or increased protein catabolism

7 We believe the loss of plasma proteins is due to increased capillary permeability and there is an indirect loss of plasma into the extravascular spaces

8 There is an increase in the thoracic duct lymph flow and a much faster mixing rate of I^{131} tagged albumin between circulating plasma and lymph

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STUDIES ON THE AMMONIA TOLERANCE CURVE IN DOGS WITH PORTACAVAL SHUNTS*

ROBERT S LEVINE AND STANLEY P RIGLER

The ammonia tolerance test reported by Eiseman[1] has provided a simple chemical method for determining the patency of surgical portacaval shunts The importance of such information in following patients of this type is obvious but the question then arises — is it possible to quantitate the results of this test? The purpose of this study is to investigate the possibility of using ammonia tolerance to determine degrees of patency of portacaval shunts in dogs

*From the William H Danforth Laboratory for Research in Surgery Department of Surgery University of Chicago

METHOD

Four normal adult mongrel dogs, ranging in weight from 12 5 to 13 8 kg were maintained on a routine laboratory diet A preoperative ammonia tolerance curve was obtained on each dog in the following manner a control sample of venous blood was withdrawn from a hind leg vein The animal was then given ammonium citrate by mouth, 0 5 gm /kg of body weight, and subsequent samples of venous blood were withdrawn at ½ hour, 1 hour, 2 hours, 3 hours, and 4 hours All samples were obtained from an unobstructed venous flow The determinations of blood ammonia nitrogen were begun within 5 minutes of the time the blood was drawn The values for blood ammonia nitrogen were determined by Bessman's modification of the method of Seligson [3]

Each dog was then subjected to an end to side portacaval shunt, the operation being performed under nembutal anesthesia and via a thoracic approach The ammonia tolerance test was repeated 10 to 14 days after operation and, again 6 weeks after operation One to 2 days after the 6 week curve was obtained, each dog was re explored through an abdominal incision, and a 3 mm Goldblatt clamp was placed on the portal vein immediately proximal to the anastomosis The clamp was left in the full open position producing an estimated 50 to 75% occlusion of the vein Operative portograms were obtained before and after application of the clamp by injecting 50% Hypaque into a portal vein tributary Ammonia

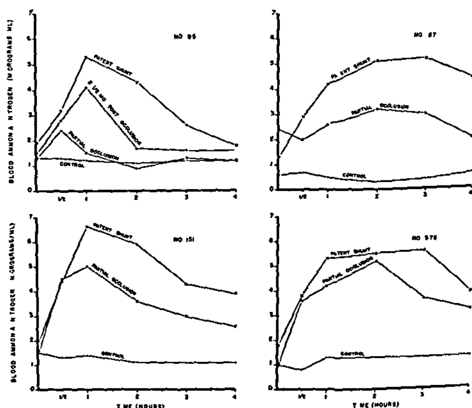


Fig 1 Ammonia tolerance curves obtained on 4 dogs before and after end to side portacaval shunt and following partial occlusion of the shunt with a Goldblatt clamp

tolerance curves were again obtained 10 to 12 days following the second operative procedure One dog, No 95, was subjected to still another ammonia tolerance curve 2½ months after application of the clamp

At the conclusion of the study, each animal was sacrificed and the circumference of the anastomosis was measured

RESULTS

The ammonia tolerance curves obtained on each dog are presented in Figure 1 It will be noted that only one of the two curves obtained before application of the Goldblatt clamp is included This is done for the sake of clarity in the graphic presentation In each case there was actually very little difference in the two curves

In every instance, the control curves were nearly flat and contained no values higher than we have observed to be normal for dogs by our methods

Following the establishment of an end to side portacaval shunt, each dog exhibited a curve which was significantly higher than the normal The peak values ranged from 5 2 ug/ml to 6 6 ug/ml and were reached in anywhere from 1 to 3 hours, depending upon the dog

After application of the Goldblatt clamps, the curves obtained on dogs No 95 and No 187 fell markedly but still showed abnormally high peak values The values for the curves obtained on dogs No 151 and No 576, however, fell only slightly

The additional curve in the case of dog No 95 shows further elevation in the direction of the fully patent shunt

At no time did any of the dogs exhibit signs of ammonia intoxication

Operative portograms taken immediately prior to the application of the Goldblatt clamp revealed all shunts to be fully patent Those taken after application of the clamp however, showed the obstruction to be marked in dogs No 95 and No 187 but only minimal in dogs No 151 and No 576 Portograms on dogs No 187 and No 151 are shown in Figure 2 and are representative of the findings in the other 2 dogs

Postmortem examinations revealed all shunts to be patent The circumference of the anastomoses ranged from 1 6 cm to 2 3 cm In dogs No 95

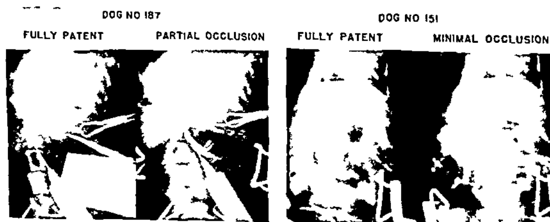


Fig 2 Operative portograms on dogs Nos 187 and 151 *demonstrate the* patency of the portacaval shunt and the degree of occlusion obtained following application of a Goldblatt clamp to the portal vein

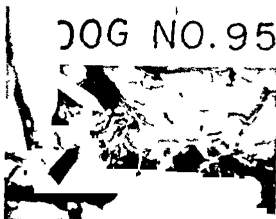


Fig 3 Large venous collaterals can be seen bypassing the Goldblatt clamp (upper arrow) and re entering the portal vein and/or the vena cava in the region of the anastomosis (lower arrow)

and No 187 the Goldblatt clamps had been accurately applied to the portal vein In dog No 151 the clamp had been applied to only one of three major tributaries of the portal vein while in No 576 it included only one of two major tributaries

The dense connective tissue mass investing the clamp in No 95 was extraordinarily rich in venous collaterals which bypassed the clamp to enter the portal vein and/or the vena cava The collaterals are shown in Figure 3

DISCUSSION

Marked elevation of the ammonia tolerance curve in the animals studied was indicative of a patent portacaval shunt This is in agreement with the findings of Eiseman and is verified by the operative portograms and post mortem findings

When the effective lumen of the shunt was decreased to at least 50 to 60% of its original size as in No 95 and No 187 a marked fall is noted in the curve In these instances the difference in the peak values of the curve before and after clamping were 2 9 and 2 2 ug/ml respectively In dogs No 151 and No 576 where considerably less than 50% occlusion was achieved these differences were *1 5 and 0 4 ug/ml respectively*

From these data it would appear that diminutions of 50% or more in the lumen of an end to side portacaval shunt could be easily recognized by employing the ammonia tolerance curve However the return of higher values in dog No 95 2½ months after clamping is at least in part indicative of the extensive venous collaterals which bypassed the clamp In view of this it is not inconceivable that in patients with extensive natural shunting as in portal hypertension an elevated ammonia tolerance curve could be obtained Therefore if the status of surgical shunts is to be followed by this method one must have in addition to a preoperative curve an early postoperative curve as a basis for comparison with subsequent tests

SUMMARY AND CONCLUSIONS

Four mongrel dogs were subjected to end to side portacaval shunts Ammonia tolerance curves were obtained before and after operation The shunts were then partially occluded and the ammonia tolerance curves repeated Patency of the shunts as well as partial occlusion were demonstrated by operative portograms

It is concluded that diminutions in the lumen of the shunt of 50% or more can easily be detected by the use of the ammonia tolerance test but that interpretation of the results could be complicated by the development of collaterals in an occluded or partially occluded shunt

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TOLERANCE OF EVISCERECTOMIZED DOGS TO EXOGENOUS AMMONIUM SALTS*

WALTER LAWRENCE, JR, ARTHUR E SCHWARTZ KATHLEEN E ROBERTS, AND HENRY T RANDALL

The pattern of blood ammonia levels following oral administration of ammonium salts has been utilized as an aid in the evaluation of patients with liver disease and is the basis for the ammonium tolerance test [1] It has been observed, however, that patients with ammonia toxicity have higher ammonia levels in the arterial blood than in peripheral veins [2] Although the liver normally plays a prominent role in ammonia removal, this arteriovenous ammonia difference suggests the presence of significant extra hepatic detoxifying mechanisms To evaluate the importance of peripheral tissues in this regard the following experiments were performed

METHOD

Eight dogs (13 to 30 kg) were subjected to ether anesthesia and one-stage abdominal evisecerectomy as described by Markowitz [3] The hepatic vena cava was replaced by a polyethylene tube and all abdominal viscera except adrenal glands were excised Bilateral nephrectomy was also carried out to eliminate any renal effect on ammonia levels Five additional dogs were subjected to one stage total hepatectomy These dehepatized dogs and two eviscerectomized dogs served as controls and received no treatment other than hourly injections of glucose (0 25 to 0 50 gm/kg)

*From the Andre and Bella Meyer Physiology Laboratories the Sloan Kettering Institute and [illegible] New York New York Supported by a grant [illegible] 9261
With th[illegible] Penecko J DeAngelis D Garms M Hood D Miller and F Washington.

Ammonium acetate dissolved in 20 cc of 5% dextrose was administered intravenously to 6 eviscerectomized dogs (23 to 153 mg /kg), over a 5 to 15 minute period Arterial blood ammonia, pH, plasma CO_2 and urea were then determined at frequent intervals by previously reported methods[4] The increase in ammonia nitrogen in the extracellular fluid was calculated from the increase in the blood ammonia level and the estimated extra cellular fluid volume In two experiments inulin space was used as an estimate of extracellular fluid volume and in the remainder of the experi ments the extracellular fluid was assumed to be 20% of body weight †

RESULTS

Control dogs. In the 2 control dogs subjected to abdominal eviscerectomy blood ammonia was maintained postoperatively at relatively low levels In contrast to these findings, the blood ammonia progressively rose in the 5 hepatectomized animals This was partially prevented by preoperative bowel preparation with neomycin in one experiment (Figure 1)

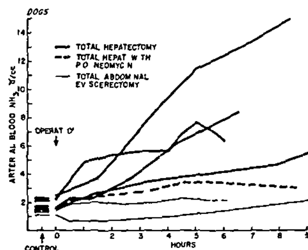


Fig 1 Arterial blood ammonia levels following total hepatectomy and ab dominal eviscerectomy

Table 1 Typical Pattern of Changes in Blood Ammonia, pH, CO_2, and Urea Following Intravenous Administration 1.5 gms Ammonium Acetate to Eviscerectomized Dog (50 mg /kg)

Dog F (30 kg) Inulin Space ≈ 3 9L		TIME (MINUTES) AFTER AMMONIUM ACETATE INJECTION			
	CONTROL	15	30	60	120
NH_3 γ/cc	1 1	7.5	5 1	1 9	1 6
% Inj NH_3 in F C F		7 6%	4 8%	0 9%	0 6%
pH	7.23	7.28	7.28	7.28	7.21
CO_2	18 9	12.5	13 1	12 7	11 6
B U N	20 1		27.2	23 6	21 9

†Plasma ammonia values in our laboratory have been slightly lower than simultaneously determined whole blood ammonia values However this difference did not significantly alter the calculations of total extracellular ammonia

The dogs subjected to total removal of the gastrointestinal tract, in addition to the liver, were therefore selected for this study This eliminated a significant unmeasured source of ammonia and it was possible to correlate changes in blood ammonia with the quantity of ammonium acetate administered

The values for pH and CO_2 indicated persistent metabolic acidosis in all dogs following operation (Table 1) Changes in blood pH during the course of each experiment were not of such magnitude as to play any role in the alterations in blood ammonia described below

Ammonium salt administration. The serial blood ammonia levels following intravenous administration of ammonium acetate to 4 of the eviscerectomized dogs (50 80 mg/kg) are shown in Figure 2 After an initial post injection rise in each experiment, a rapid fall in blood ammonia occurred over a 2 to 4 hour period A similar disappearance of ammonia was observed after repeated injection of a smaller quantity (Fig 3) After injection of a larger quantity in one dog (153 mg/kg) the subsequent fall in blood ammonia was similar, but less profound, and persistent coma

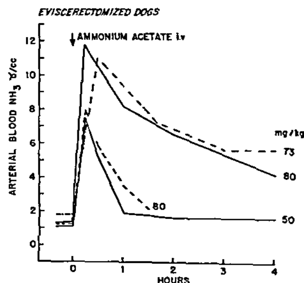


Fig 2 Arterial blood ammonia following intravenous administration of ammonium acetate (50 80 mg/kg) to 4 eviscerectomized dogs

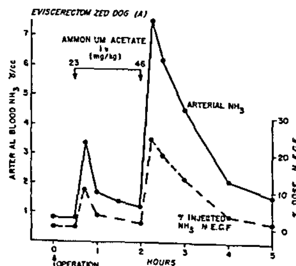


Fig 3 Arterial blood ammonia following repeated administration of ammonium acetate to eviscerectomized dog

occurred following injection There was no significant arteriovenous ammonia difference demonstrated in 3 dogs in which simultaneous measurements were made and there was no significant change in blood urea (Table I)

Two to 4 hours after ammonium acetate administration (23 to 80 mg/kg) only 0 6% to 5% of this ammonia nitrogen could be accounted for in the extracellular fluid (Table 2) Two hours after the larger apparently fatal injection (153 mg/kg) only 7 2% of the injected ammonia nitrogen could be accounted for in the extracellular fluid

Table 2 Total Extracellular Fluid Ammonia 2 and 4 Hours Following Administration of Ammonium Acetate to Eviscerectomized Dogs

DOG	AMMONIUM ACETATE (MG/KG) DOSE	EXTRACELLULAR FLUID (LITERS)	% INJECTED NH_4 IN E C F 2 HRS	4 HRS
A	23 (1st dose)	2 6	1 6%	
	46 (2nd dose)			1 6%
B	78	2 6	1 0%	
C	78	3 8		5%
D	153	2 6	7 2%††	
E	80	1 7†		2 4%
F	50	3 9†	0 6%	

† = Inulin Space
†† = Expired

DISCUSSION

The blood ammonia curves following ammonium salt administration to eviscerectomized dogs bore a striking similarity to the ammonia curves reported in patients with intact livers by White *et al*[1] The quantity of ammonia nitrogen administered in relation to body weight of the human subjects as reported by these investigators was in the same range as the dosage used in the experiments reported here There is no question that the liver is a major site of removal of exogenous ammonia but the similarity of disappearance curves in eviscerectomized animals raises strong objections to the use of ammonium tolerance curves for clinical evaluation of liver function

Estimates of total extracellular fluid ammonia could only be approximate even when inulin space measurements were carried out However the fact that only 0 6% to 5% of the injected ammonia nitrogen could be accounted for in the extracellular fluid of all but one of the dogs seems highly significant An unequal distribution of total ammonia between intracellular and extracellular compartments at equilibrium is undoubtedly partially responsible for these low values for total extracellular ammonia The distribution of total ammonia between the intracellular and extra

cellular space, in terms of concentration, has been reported to be roughly 4 1 † Since the intracellular water is approximately 2 times the volume of extracellular water, we would expect the total intracellular ammonia to be approximately 8 times the total extracellular ammonia Even considering the intracellular extracellular distribution of ammonia as 8 1, we could account by calculation for only 5% to 40% of the ammonia administered to these dogs The curves of decreasing blood ammonia following ammonium acetate administration are, therefore, a graphic representation of the net result of intracellular transfer of ammonia, and ammonia removal by metabolic processes Since intracellular transfer of ammonia can account at most for only a fraction of that removed, the peripheral detoxification mechanisms assume real significance

Bessman and Bessman[2] reported a peripheral arteriovenous ammonia difference in patients with ammonia toxicity The significance of the peripheral tissues as a site of ammonia removal is emphasized by our findings Flock, *et al*,[7] studied amino acid concentrations after total hepatectomy in dogs and demonstrated a progressive increase in glutamine and a progressive decrease in glutamic acid concentration in muscle In view of the established effectiveness of glutamic acid in lowering blood ammonia in patients, these tissue changes suggest a peripheral mechanism for this progressive reduction in blood ammonia

SUMMARY

Ammonium acetate was injected intravenously in abdominally eviscerectomized dogs An initial rise in blood ammonia occurred and this was followed by a progressive fall toward control values In all but one of the animals studied only 0 6% to 5% of the injected ammonia could be accounted for in the extracellular fluid 2 and 4 hours after administration of the ammonium salt These findings confirm previous work[2] that suggested peripheral detoxifying mechanisms for ammonia This should influence the interpretations of the "ammonium tolerance tests and seriously limits their value as a measure of liver function

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†At equilibrium intracellular and extracellular PNH_3 must be equal if passage of ammonia across cell membranes occurs as the free ammonia form The proportion of total ammonia present as free ammonia (or PNH_3) varies as a function of pH and temperature (Factors for calculating free ammonia from total ammonia concentration have been described by Jacquez *et al*[5]) If we assume a pH gradient of 0 6 across the cell membrane the more acid intracellular space would require almost four times the total ammonia concentration found in the *extracellular fluid* to maintain the equal free ammonia concentrations in these compartments These theoretical calculations of ammonia distribution corroborate the direct measurements of Christenson *et al*[6]

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We are indebted to J A Jacquez MD P Vanamee MD and J W Poppell MD for invaluable advice and criticism

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THE AMELIORATION OF EXPERIMENTAL ASCITES BY HEPATOPEXY*

ANDREW A GAGE ROBERT W MCGRATH MICHAEL J GIANTURCO AND CARLOS G SANTORO

Exudation of lymph from the surface of the liver has been shown to be the cause of ascites which follows experimental constriction of the inferior vena cava above the diaphragm That the same factor may contribute to ascites formation in cirrhosis is suggested by recent injection studies demonstrating the venous outflow block in the cirrhotic liver Obliteration of the liver surface might prevent or ameliorate such ascites The effect on experimental ascites of enclosing the liver in dense adhesions thus isolating it from the free peritoneal cavity was studied in the following experiments

METHOD

Ascites was produced in dogs by placing an aluminum band around the inferior vena cava above the diaphragm reducing its lumen by approximately 75% Lesser degrees of occlusion do not consistently produce ascites while greater interference with flow often causes death of the animal Massive adhesions to the liver were produced by mechanical abrasion of its free surface and of the adjacent portions of the diaphragm From 6 to 12 gm of talcum powder were applied to the raw areas to increase formation of adhesions

The animals were divided into three groups In the first group caval obstruction only was produced in order to observe the natural course of the ascites In the second group ascites was induced and followed later by hepatopexy and in the third group hepatopexy was performed prophylactically before ascites was induced Hemoglobin hematocrit serum protein and liver function tests were obtained at suitable intervals After a period of observation all animals were sacrificed and gross pathologic changes were noted In some instances histological examinations of pertinent organs were made

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RESULTS

Control Group In the first group of 10 dogs, the immediate effect of constriction of the inferior vena cava was an elevation of the portal and hind leg venous pressure Ascites became evident about 2 weeks after placement of the band, increasing in amount thereafter, until abdominal distention became marked in about 4 weeks During the early stages of ascites, the health of the animal seemed unimpaired but later, nutritional deficiency became apparent Hypoalbuminemia was observed after the ascites progressed but there was no alteration of liver function

Most dogs remained active throughout the experiment in spite of ascites The ascites persisted and slowly increased during the observation period of about 4 months and the fluid would re accumulate after paracentesis The abdominal subcutaneous veins became prominent early and enlargement of the deeper collateral vessels between portal and systemic veins was demonstrable by portal venography

At the time of sacrifice, the quantity of ascitic fluid ranged from a minimum of 1,200 cc in a 15 pound dog to a maximum of 13,500 cc. in a 59 pound dog In the average dog, about 5,000 cc of a clear yellowish fluid was found No peritoneal adhesions were present The liver was dark red with plaques of fibrinous exudate on the surface On section, marked venous congestion was noted and histologic examination demonstrated venous engorgement, dilated sinusoids and dilatation of the lymphatics, especially in the capsule

Ascitic Dogs Subjected to Hepatopexy. In the second group of 10 dogs, ascites was induced and the animals later were subjected to hepatic abrasion and poudrage Of the 10 dogs, 6 failed to demonstrate any ascites subsequent to hepatopexy Hypoalbuminemia improved slowly because ascitic fluid was no longer being formed When the animals were sacrificed from 2 to 3 months later, no ascites was present The liver was encased in adhesions about 2 mm thick Dilated lymphatic vessels were present on the pleural surface of the diaphragm In two dogs the livers appeared normal in contrast to their earlier congested appearance at the time of hepatopexy

In the remaining 4 dogs, ascitic fluid continued to accumulate following hepatopexy although in much smaller amounts than preoperatively These animals were operated upon again and remaining patches of free liver

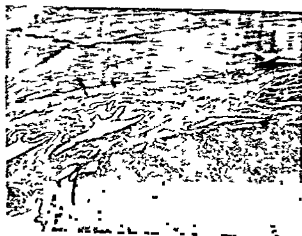


Fig 1 Photomicrograph (X44) of the adhesions between the diaphragm and liver following hepatopexy for ascites showing numerous dilated lymphatic vessels

surface were abraded This eliminated the ascites in 2 dogs The other 2 still formed a small amount of fluid but they were markedly improved since at the time of sacrifice only 500 and 900 cc respectively were present whereas each animal s peritoneal cavity contained about 6 000 cc of fluid before the first hepatopexy A cure might have been obtained by further obliteration of the liver surface

Hepatopexy Performed Prior to Constriction of the Inferior Vena Cava In the third group of 10 dogs hepatopexy was performed prior to the induction of ascites In most of the dogs the adequacy of the hepatopexy was checked at a second laparatomy and any remaining free areas of liver surface were abraded When the animals had recovered from the opera tion(s) the inferior vena cava was constricted in the same manner and degree as in the control group None of these dogs developed ascites They remained healthy and active with a stable weight and no significant changes in the laboratory tests were observed At the time of sacrifice the livers were encased in adhesions On section the livers in 8 dogs were dark red and congested but in 2 dogs they appeared grossly normal No ascites was present though the caval obstruction was satisfactory

DISCUSSION

These experiments again confirm the importance of lymph exudation from the liver in the formation of ascites produced experimentally by partial occlusion of the hepatic venous outflow The benefits of hepatopexy appear to be due to isolation of the liver from the free peritoneal cavity where lymph could collect The degree of benefit is related to the extent of obliteration of the free liver surface When obliteration is complete no ascitic fluid is formed

No alteration of liver function tests was noted but none could be ex pected since this experiment produces only a congested weeping liver and not a cirrhotic one Hypoalbuminemia is proportionate to the loss of albumin with the ascitic fluid and can be corrected if this loss is prevented or abolished by hepatopexy

The applicability of this procedure to the patient with ascites due to cirrhosis remains to be proved Obviously the cirrhotic liver is quite dif ferent from the congested liver produced in this experiment However there is some similarity with regard to the pathogenesis of ascites since in both conditions there is interference with hepatic venous drainage Madden and co-workers[1] have demonstrated by postmortem injection studies that obstruction in the hepatic venous outflow while perhaps not the sole cause is at least a major contributory factor in the formation of cirrhotic ascites They suggested hepatopexy as a treatment for ascites in the cirrhotic patient postulating that collateral veins may form in the adhesions increasing the hepatic venous outflow

Our experiments demonstrate that experimental ascites can be controlled by hepatopexy but suggest a different explanation for its mode of action Regardless of the latter the operation is sufficiently effective to warrant a clinical trial We have used hepatopexy on a few cirrhotic patients with irreversible ascites Our experience is too limited to permit any conclusions however the results warrant further trial

SUMMARY

Abrasion and poudrage of the liver surface was effective in controlling ascites produced by partial occlusion of the inferior vena cava above the diaphragm. Isolation of the liver from the free peritoneal cavity, preventing the accumulation of lymph, appears to be the mechanism of its efficacy.

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Pancreatitis

THE CLINICAL PICTURE OF THE SEQUENTIAL DEVELOPMENT OF ACUTE HEMORRHAGIC PANCREATITIS IN THE DOG*

ROBERT B PFEFFER ORKAN STASIOR AND J WILLIAM HINTON

In spite of much experimental work, the etiology of acute hemorrhagic *pancreatitis remains obscure Although certain predisposing factors have* emerged from these studies they are difficult to assess This is due in great part to the fact that the lesion has been produced by so many varying techniques In addition there has been no correlation at periodic intervals of the final pathologic process with the sequential development of hemorrhagic pancreatitis Therefore even though the lesion may have been produced differently in each instance, the events leading to its production may have been remarkably similar

This investigation is aimed at hourly observations of both the gross and microscopic lesion of the pancreas and a correlation with the clinical course and laboratory findings in a series of dogs One of the basic problems in a study of this kind is to create a situation in which acute hemorrhagic pancreatitis is produced with routine consistency in a similar manner A second important fundamental is that the pancreas undergo a minimum of handling and surgical trauma in the establishment of the process Both of these criteria were met in the creation of a closed duodenal loop obstruction in the dog To our knowledge acute hemorrhagic pancreatitis has not been created in this manner before

METHOD

Healthy processed mongrel dogs weighing 11 to 20 kg were used The animals were fasted for 24 hours preoperatively and given 600 000 u of penicillin Using sterile technique the abdomen was entered through an upper midline incision A closed duodenal loop obstruction was created by dividing the duodenum just beyond the pylorus and again beyond the reflection of the pancreas onto the dorsal mesentery This made a loop approximately 7 to 10 cm in length into which the pancreatic ducts drained Bile was completely eliminated from the loop by dividing and ligating the common bile duct near its entrance into the duodenum (Figure 1) The loop was irrigated with 150 cc sterile saline prior to closure and cultures were taken Gastrointestinal continuity was reestablished by a gastroduodenostomy

Serum amylase and lipase values were obtained preoperatively and at autopsy In addition amylase and lipase studies were performed on the fluid in the duodenal loop and peritoneal fluid when it appeared The

*From the Department of Surgery New York University Post Graduate Medical School New York N Y Aided by grants from the U S Public Health Service (2 CC 110) the John A Hartford Foundation and the Samuel H Kress Foundation

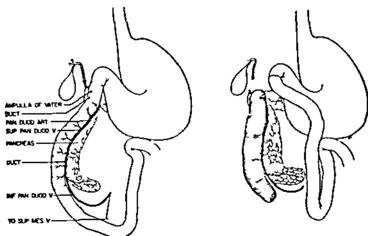


Fig 1 The normal anatomy in the dog and the creation of a closed duodenal loop

dogs were sacrificed with an overdose of intravenous nembutal anesthesia at hourly intervals Serial photographs and histologic sections were made of each animal so as to record the progression of the process The amylase activity was determined by the method of Norby as modified by Agren and Lagerlof[1] Lipase values were obtained by the method of Cherry and Crandall[2]

RESULTS

The earliest gross sign of a developing pancreatitis was evident in 4 hours Edema was present in the head and body of the gland There was little change in this process during the next 4 hours but at 9 hours a small area of hemorrhage appeared in the head of the pancreas From this point the progression was rapid At 10 hours the head and body were enveloped in the advancing reaction Finally in 11 hours the entire pancreas was hemorrhagic No fat necrosis was present in any of the animals

Histologic sections were confirmatory As early as 4 hours foci of extravasated blood were scattered through pancreatic parenchyma prior to evidence of necrosis With the passage of time foci of parenchymal necrosis appeared During the entire developmental stages the pancreatic ducts remained intact with a normal mucosa and duct wall Arterial vessel walls showed no early evidence of necrosis only the capillaries and thin walled veins were congested and in some areas thrombosed Foci of extravasated blood and parenchymal necrosis persisted to 9 hours when suddenly a rapid extensive progressive hemorrhagic extravasation and parenchymal destruction occurred to involve large areas of the pancreas In this period no inflammatory response was demonstrable

Serum amylase and lipase levels were graphically illustrated (Figure 2) The normal values in the dog are from 2 000 to 4 000 mg % and the hourly rise to pathologic levels were clearly indicated Of somewhat greater interest was the appearance of 30 cc of hemorrhagic peritoneal fluid at 4 hours concomitant with the development of a microscopic lesion in the pancreas The volume of this fluid consistently increased to 125 cc at 11 hours Amylase activity in the peritoneal fluid was always greater than in the serum The appearance of this hemorrhagic peritoneal fluid 7 hours before the marked rise in serum amylase makes its presence of diagnostic importance (Figure 3)

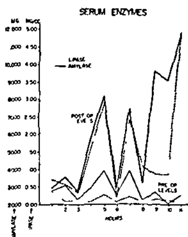


Fig 2

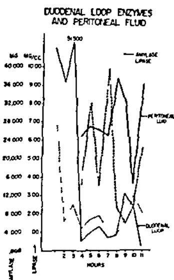


Fig 3

Cultures of the duodenal loop pre-operatively were negative except for Clostridia Welchi on two occasions Postoperative cultures in the 11 hour dog revealed E Coli, non hemolytic streptococus and a member of the clostridium group

Finally the fate of the closed duodenal loop should be mentioned Concomitant with the developing hemorrhagic pancreatitis there was an increasing distention of the duodenum The amount of fluid recovered from the closed loop advanced from 30 cc at 1 hour to 85 cc at 11 hours Gangrene of the duodenum was never present Since the duodenum and the head and body of the pancreas share a common blood supply via the superior pancreaticoduodenal artery, the duodenum serves as an excellent monitor that no interference with the major blood supply has occurred

DISCUSSION AND CONCLUSIONS

Acute hemorrhagic pancreatitis has been produced throughout the entire pancreas in a period of 11 hours by means of a closed duodenal loop obstruction Bile was completely eliminated as a factor by dividing and ligating the common bile duct The etiology of the lesion created here seems to be primarily vascular in nature, dependent on the congestion and occlusion of small blood vessels

Increasing distension of a closed duodenal loop can occlude capillaries and venules of the dog at relatively low pressures [3] The first microscopic lesion which can be seen is an extravasation of the blood into the parenchyma with a congestion of small blood vessels Foci of intralobular necrosis appear 1 to 3 hours later Further support for this theory is the fact that the hemorrhagic process appears initially in the head and body of the pancreas The uncinate process and tail of the pancreas are secondarily affected since each has its own blood source via the superior mesenteric and splenic arteries The role which ductal obstruction might play in conjunction with the vascular lesion must be assessed Certainly this lesion cannot be produced by ductal obstruction alone [4] Only tissue *devoid of blood supply could present the rapid development of such wide*

spread pancreatic parenchymal hemorrhage and necrosis without a concomitant inflammatory response

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A STUDY OF THE RELATIONSHIPS BETWEEN ALCOHOLIC INTOXICATION VOMITING AND ACUTE HEMORRHAGIC PANCREATITIS*

ANTONIO BOBA, ARTHUR A STEIN, YOSHIHIKO NAKAMURA, AND SAMUEL R POWERS, JR

It has been observed clinically that pancreatitis frequently follows the ingestion of large quantities of alcohol This report presents a series of experiments concerning the possible role of alcohol as a factor in the development of acute pancreatitis The development of acute hemorrhagic pancreatitis in dogs following the construction of a common pancreatic and biliary channel has been reported[1] It was suggested that the development of pancreatitis resulted from the activation of proteolytic enzymes in an actively secreting pancreas and that this activation was due in these cases to reflux of bile into the pancreatic duct In a series of preliminary experiments, alcohol was fed to 4 animals with prepared common channels Pancreatitis resulted in each case when vomiting was induced by the injection of apomorphine

The presence of gastric juice in the duodenum is known to be a powerful stimulant to pancreatic secretion A pyloromyotomy was therefore performed in 4 animals to more readily allow flow of gastric juice and alcohol into the duodenum These animals with a common channel and pyloromyotomy developed fulminating fatal hemorrhagic pancreatitis Only one animal survived long enough to receive the alcohol feedings

Since bile, acid, and alcohol were all present in the duodenum of animals developing pancreatitis, a series of experiments were designed to test the significance of each factor alone or in combination in the pathogenesis of acute pancreatitis

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*From the Departments of Experimental Surgery and Pathology The Albany Medical College of Union University Supported in part by a *grant* (RG 4420) from the Division of Research Grants Public Health Service

METHOD

There are seven possible combinations of one or more of these three factors Each factor was given a code letter and each experiment could then be described by a unique combination of the three letters (Table IA) In order to separately study these factors, a series of 14 animals was prepared by forming a duodenostomy utilizing a polyethylene tube which fitted a #15 gauge needle This allowed the direct injection of the test materials into the duodenum

All animals were sacrificed or died 12 hours following the completion of each experiment and careful postmortem examination was performed In these experiments the normal entry of the bile and pancreatic ducts was not disturbed

The development of acute pancreatitis was ascertained by histologic study *The diagnosis of acute pancreatitis was confined to those animals* where the pancreas showed interstitial hemorrhage, fat, or pancreatic parenchymal necrosis Interstitial edema alone was not classified as acute pancreatitis

Group A. In 3 dogs, 100 ml of saline was introduced into the duodenum without ill effect Twenty minutes later 6 0 mg of apomorphine were injected subcutaneously and in all cases violent retching and vomiting was produced This procedure was repeated 4 times in a 24 hour period and the animal was sacrificed 12 hours after the last feeding None of these animals showed acute pancreatitis

Group B. In 3 animals, the experiment was repeated in an identical manner, except that 100 ml of 50% alcohol was substituted for saline At sacrifice, a severe duodenitis and pancreatic septal edema was found *Fat and parenchymal necrosis did not occur*

Group C. When 100 ml of 0 1 N hydrochloric acid were substituted for saline, in the majority of cases spontaneous vomiting immediately followed the instillation In all four cases, severe hemorrhagic pancreatitis *could be demonstrated histologically In one animal the polyethylene* cannula was left in place for a period of time equal to that of an average experiment The pancreas was histologically normal at sacrifice

Group D. Since bile was present in the duodenum in all of the above experiments, an additional group of animals was studied in which bile was diverted from the duodenum by means of Roux Y *choledochojejunostomy* A simultaneous pyloromyotomy was performed to insure free flow of gastric acid into the duodenum In 5 such animals the feeding of alcohol followed by apomorphine injection failed to produce histologic evidence of pancreatitis

Group E. The factor of gastric acidity was completely eliminated in a group of 3 animals by performing a Billroth II subtotal gastrectomy Alcohol feedings followed by apomorphine injections had no deleterious effect upon the pancreas

Group F. The above studies indicate that the presence of gastric acid in the duodenum is an important factor in the pathogenesis of acute pancreatitis In order to facilitate the flow of gastric juice into the duodenum, pyloromyotomy was carried out in a group of 9 animals Five of these *animals were fed alcohol and the remaining 4 were fed saline* Each feeding

Table 1

A			B		
a = bile b = acid c = alcohol	FACTORS a b c	EXPERIMENT GROUP	PANCREATITIS	NO PANCREATITIS	NOT CARRIED OUT
	0 0 0		1 1 0	1 0 0	0 0 0
	1 0 0	A	1 1 1	0 1 1	0 0 1
	0 1 0	D		1 0 1	
	0 0 1			0 1 0	
	1 1 0	C			
	0 1 1	D			
	1 0 1	E B			
	1 1 1	F			

LEGEND A The three factors of bile acid and alcohol listed in all possible combinations with the experiment group which each combination uniquely describes
B *Results obtained in each combination*

was followed by the administration of apomorphine All of these animals showed septal edema and focal hemorrhage without fat necrosis In 6 control animals with intact pyloric sphincter, the ingestion of alcohol followed by vomiting produced no incidence of acute pancreatitis

Group G A series of experiments were carried out where the vomiting factor was completely eliminated In 3 dogs, smaller amounts, 40 to 60 ml of 0 1 N hydrochloric acid were instilled very slowly in the duodenum via a catheter duodenostomy No vomiting was produced by this injection and the apomorphine was withheld After 4 such feedings in 24 hours, and 12 hours after the last feeding, the animals were sacrificed and the pancreas examined In one case no conclusive diagnosis could be made, but in the other two, interstitial pancreatitis with focal hemorrhage was seen It is interesting to observe, however, that no fat necrosis could be detected, whereas, this was an obvious feature in the animals where vomiting followed the acid instillation

DISCUSSION

The above experiments, suitably coded in terms of the factors under study, are shown in Table 1B It can be seen that pancreatitis will develop if, and only if, acid and bile are present in the duodenum It is interesting that in all possible combinations of factors where bile and acid do not occur together, pancreatitis does not develop It is also apparent from these studies that alcohol has no inherent specific capacity to induce acute pancreatitis

It is known clinically that spontaneous pancreatitis may appear in any patient although it will show a relative predilection for consumers of large amounts of alcoholic beverages Our results would tend to relegate the ingestion of alcohol to a secondary role When, however, the results were

evaluated from a quantitative, rather than from a qualitative view point it was found that the most severe form of pancreatitis was seen after relatively large amounts of acid entered the duodenum and vomiting then ensued *(Group C), or when a large quantity of bile could enter the pancreas* via a common channel and pancreatic secretion was stimulated as a result of pyloromyotomy

The ingestion of alcohol in humans will stimulate both gastric secretory activity and vomiting and it is likely that the proper role of alcoholic intoxication in the pathogenesis of spontaneous clinical pancreatitis is as a stimulating or potentiating factor Alcohol would therefore seem to act in two ways first, by stimulating the production of gastric acid, which on contact with the duodenal mucosa, would result in increased secretory activity of the pancreas, and second, by inducing vomiting which would force bile from the duodenum into the pancreatic ducts

It may be concluded from the present study that in the intact dog the simultaneous presence of bile and acid in the duodenum is a necessary condition to the development of pancreatitis The severity of the pancreatitis will in turn be regulated by the amount of acid and the presence or lack of vomiting *In cases where a common channel* for pancreatic and biliary secretions had been artificially created and bile could readily pass into the pancreas, increased flow of acid into the duodenum was a strong potentiating factor

Alcohol may *therefore* play *a role in the pathogenesis of acute pancreatitis* by stimulation of gastric acid secretion and by the induction of vomiting

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HEMOLYTIC ASPECTS OF ACUTE PANCREATITIS*

FRANK E BERRIDGE, JR , AND ROBERT N WATMAN

The purpose of this study was to find some mechanism which might explain the excessive red cell mass depletion accompanying pancreatitis A series of dogs was subjected to pancreatitis or trypsin infusion in order to evaluate the resultant changes in red cell integrity

METHOD

The dogs used were healthy mongrels of both sexes and weighed between 10 and 14 kg The animals were first anesthetized by intravenous administration of sodium pentobarbital Experimental pancreatitis was produced by transduodenal retrograde injection of 5 ml of autogenous bile into the accessory pancreatic duct with the main pancreatic duct temporarily occluded to prevent regurgitation This injection was of sufficient force to cause an observable, diffuse extravasation of bile within the pancreatic parenchyma As an operative control some dogs underwent duodenotomy with cannulization of the pancreatic duct but without the injection of bile Infusion of 250 ml solution of sterile normal saline containing commercially prepared crystalline trypsin (1 000 Armour units per mg) was administered over a 2 hour period into a peripheral vein of other dogs Blood samples were collected from the jugular vein prior to and 4, 6, and 8 hours after completion of the procedure

Erythrocyte osmotic fragility studies were performed on potassium oxalated blood which was collected with sterile precaution and incubated at 37°C for 24 hours One drop of blood was added to serial saline dilutions, ranging from physiologic saline to distilled water in increments of 0 036 gm % NaCl and allowed to stand at room temperature for 2 hours before reading

Employing the technical precautions of Cockrell and Naumann,[1] 10 ml of blood was drawn into a syringe containing 1 0 ml of heparin sodium solution (10 mg heparin) The method of Shinowara[2] was used with a Beckman spectrophotometer to determine the plasma hemoglobin

RESULTS

A simple index of red cell membrane integrity can be derived from osmotic fragility Although minimal changes in fragility were detected by immediately exposing blood to serial dilutions of saline, marked changes were observed if the specimens were first incubated as proposed by Young[3] Figure 1 depicts the results in the pancreatitis series as compared to the control group The ordinate corresponds to the dilution of saline at which beginning hemolysis was observed In order to exclude bile as a cause for this increased fragility, 5 ml of bile was injected directly intravenously in a series of dogs but no observable change occurred after 4 hours had elapsed

Free plasma hemoglobin serves as an index of erythrocyte destruction

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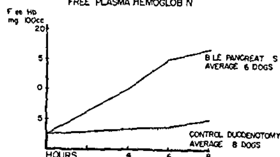


Fig 1



Fig 2

occurring simultaneously with increased fragility Figure 2 illustrates the level found in the pancreatitis and duodenotomy groups

To test the hypothesis that circulating trypsin might be responsible for these alterations during pancreatitis 250 mg and 65 mg of trypsin were infused Marked increases of fragility and plasma hemoglobin were seen with the former dose but no change followed the latter dosage The results obtained after infusion of 125 mg of trypsin in a series of 5 dogs are shown in Figure 3 for incubated osmotic fragility and in Figure 4 for free plasma hemoglobin

In addition oxalated blood samples were obtained from 6 patients during attacks of pancreatitis (serum amylase in excess of 700 Somogyi units) In the five acute cases marked hemolysis was evident in plasma after 24 hours incubation at body temperature An osmotic fragility of 0 792 gm % NaCl was obtained for the remaining patient who had chronic pancreatitis The fragility was 0 684 gm % NaCl for these patients after recovery

DISCUSSION

Only a portion of the marked red cell mass depletion associated with acute pancreatitis can be attributed to the intrapancreatic extravasation of blood[4] It was felt that the inflammed pancreas might release circulating enzymes which were instrumental in lysing the erythrocyte Both trypsin and lipase are capable of increasing the permeability of the red cell mem

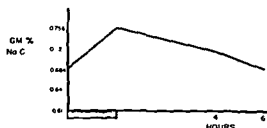


Fig 3

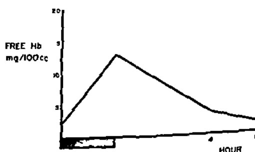


Fig 4

brane *in vitro*[5] The degree of damage to the erythrocyte varies with the enzyme its concentration and the duration of exposure Measurement of blood trypsin during pancreatitis has not been entirely successful because of the presence of antifibrinolysin but Elliott[6] has demonstrated that con siderable proteolytic activity exists in the plasma during this disease

The alterations in red cell integrity following pancreatitis resemble those following trypsin infusion in 125 mg doses The early decline in fragility and plasma hemoglobin for the trypsin infused disease may possibly be explained by its brief sublethal character and the lack of other enzymes such as lipase

The premature autolysis of incubated human blood samples obtained during attacks of pancreatitis bears further study

SUMMARY

An increased erythrocyte membrane permeability as detected by osmotic fragility testing occurs simultaneously with red cell destruction as evidenced by free plasma hemoglobin in experimental pancreatitis Similar changes occur following the infusion intravenously of small amounts of trypsin This suggests that the action of trypsin upon the erythrocyte offers a pos sible and probable explanation for a portion of the red cell mass depletion accompanying pancreatitis

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THE EFFECT OF PROPYLTHIOURACIL ON ACUTE HEMORRHAGIC PANCREATITIS IN DOGS*

ROBERT E PAULETTE, THOMAS W CHALLIS, L CORSAN REID AND J WILLIAM HINTON

The clinical use of propylthiouracil in the treatment of acute pancreatitis was first reported by Starr[1] at the Pan Pacific Conference in 1954 It was observed that patients with known pancreatitis were aggravated with thyroid preparations and alleviated with oral propylthiouracil Reid *et al*[2 3 4] have shown that propylthiouracil is not simply an antithyroid drug, but exerts its action on all tissues and organs by inhibiting oxygen utilization With less oxidative activity within the tissues, less energy is released and so more cellular energy is conserved to maintain cellular and organ integrity

The necrosis of the pancreas is due to the destruction of cellular structure, thus anything that would delay cellular necrosis should be of therapeutic benefit It was postulated that the inhibition of oxygen uptake by n propyl thiouracil would reduce the metabolic activity of the pancreas so that it would be placed at partial rest By so doing, the energy of the gland would be available to maintain cellular integrity rather than functional activity

METHOD

The initial problem in testing the hypothesis was to find a reliable way of producing acute hemorrhagic pancreatitis Methods used included (A) pressure injection of bile into the main pancreatic duct (B) method 'A plus complete ligation of the duct and (C) method 'B' plus blunt trauma to the pancreatic parenchyma These were all found to be unreliable A reliable method which was employed has recently been described by Pfeffer *et al*[5] The abdomen is opened in the midline and the pylorus is mobilized and excised The upper end of the duodenum is closed The duodenum is then transected below the main pancreatic duct and the proximal end closed creating an isolated closed duodenal segment The common bile duct is divided and ligated to exclude bile as an etiologic agent The continuity of the intestinal tract is reestablished with a gastroduodenostomy

Twenty five mongrel dogs were prepared in the above manner, 13 were given n propylthiouracil and 12 were given 500 cc of saline intravenously and kennel water Each of the 13 dogs receiving propythiouracil were given 1,000 mg by mouth daily for 2 days preoperatively, 500 mg in 500 cc of normal saline intravenously during the operation, 500 mg in 250 cc saline intravenously at 8 and 16 hours postoperatively The animals were sacrificed and autopsied at 24 hours

RESULTS

In the control group, 11 of the 12 dogs appeared grossly to have acute pancreatitis with marked hemorrhage and necrosis of the pancreas Fat necrosis was present about the gland and in widely scattered areas of the

*From the Department of Surgery New York University Post Graduate Medical School New York, N Y Supported by grants from the John A Hartford Foundation and the Samuel H Kress Foundation

mesentery and omentum and occasionally pericardium The twelfth dog had an edematous gland with the suggestion of punctate areas of hemorrhage on cut section Each case had from 100 to 250 cc. of reddish brown fluid within the peritoneal cavity The closed loop had no leaks and was distended with an average of 60 cc of sterile fluid

In the group given propylthiouracil, 8 of the 13 had no gross evidence of pancreatitis other than slight edema and slight congestion Five animals appeared to have acute hemorrhagic pancreatitis with areas of glandular necrosis and scattered fat necrosis but with one exception, these changes were much less extensive than in the controls

Microscopically, the lesions were classified into 4 groups Group A, acute interstitial, Group B, acute hemorrhagic, Group C, combination of interstitial and hemorrhagic, and Group D, acute necrotizing pancreatitis In Group A there was infiltration of the interlobular spaces with neutrophiles, edema and at times, acute hemorrhage The parenchymal cells were not involved In Group B, the picture was one of partial necrosis of the pancreatic parenchyma with hemorrhage within the acini and occasional fat necrosis Group C was a combination of the features of groups A and B with infiltration of the interlobular spaces along with foci of necrosis and hemorrhage within the acini In Group D, the pancreas was completely destroyed leaving an indistinguishable mass of necrotic amorphous material characterized by a complete lack of inflammatory response

In the control group, microscopically 2 dogs had a marked acute interstitial pancreatitis, 5 had acute hemorrhagic pancreatitis, 3 showed a combination, and 2 dogs showed acute necrotizing pancreatitis

In the group treated with propylthiouracil 6 dogs had a mild interstitial pancreatitis, 3 had acute hemorrhagic pancreatitis, 2 showed a combination and 1 showed acute necrotizing pancreatitis In this group the pancreas of 1 dog was not examined due to a marked autolysis following death before the 21 hours With one exception, all the lesions in this group showed a more moderate and less widespread histologic involvement than the controls even though both are similarly classified The exception was another animal which expired before the 21 hour limit and which showed an acute hemorrhagic pancreatitis of the same degree as observed in the control series

Five of the 25 animals died before the arbitrary 21 hour time limit, 3 in the control group and 2 receiving propylthiouracil

Figure 1 shows the hemorrhagic pancreatitis that developed in the untreated dogs at 21 hours Figure 2 illustrates the modifying influence of propylthiouracil, the pancreas appearing grossly normal The histological difference between the two groups is shown in Figure 3

DISCUSSION

It was evident from gross examination of the pancreas at 21 hours that propylthiouracil had modified the pathological picture as seen in the untreated dogs In 8 of the treated animals, acute pancreatitis was not present Microscopic examination confirmed the above findings, the only changes being slight edema In 11 of the 12 dogs treated with propylthiouracil, the pathogenesis of acute pancreatitis was delayed so that a

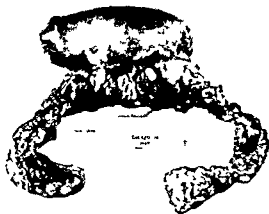


Fig 1 Isolated segment of duodenum and pancreas 21 hours postoperatively in the untreated dog showing a marked hemorrhagic pancreatitis



Fig 2 Isolated segment of duodenum and pancreas 21 hours postoperatively in the dog receiving n propylthiouracil showing a distended duodenum and grossly normal pancreas



Fig 3 Histology of the pancreas of the treated dog on the left showing normal pancreas the untreated dog on the right shows a marked hemorrhagic pancreatitis

treated pancreas at 21 hours might appear comparable to an *untreated* at 2 5 or 11 hours

It was not the object of this experiment to determine the effect of the drug on survival time One questions the validity in determining this because of the method used to produce the hemorrhagic pancreatitis However it seems reasonable to assume that anything that delays cellular necrosis will prolong the survival time, other factors excluded

The results strongly suggest that propylthiouracil delays the progressive changes seen in the pathogenesis of acute hemorrhagic and necrotic pancreatitis in dogs The preservation of cellular integrity in the pancreas is more important than any other organ because it has been shown that the intracellular pancreatic enzymes are more destructive to the pancreas itself and other tissues than those of any other organ [2]

SUMMARY

Twenty five mongrel dogs were operated upon to produce acute hemorrhagic pancreatitis and sacrificed at 21 hours Twelve dogs were untreated and developed the lesion Thirteen dogs were given 6 n propyl 2 thiouracil

(Lederle) orally and intravenously, 8 of these did not develop the lesion, 3 showed a delay in the pathogenesis of the lesion, and 2 were not protected

The results show that n propylthiouracil delays and minimizes the lesion in experimentally produced acute hemorrhagic pancreatitis in dogs

It is suggested that the therapeutic benefits in humans is due, in part at least, to placing the pancreas at rest by the inhibition of the oxidative energy release process

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THE EXCRETION OF ANTIBIOTICS IN PANCREATIC FLUID*

BERNARD GERBER, MILTON SILVERMAN AND FREDERICK W PRESTON

This report presents a study of the excretion of erythromycin, streptomycin and chloramphenicol in pancreatic fluid of a patient with chronic relapsing pancreatitis

METHOD

A 35 year old white male alcoholic had had six attacks of severe epigastric pain accompanied by nausea and vomiting and had lost thirty pounds in a 12 month period He had diabetes mellitus There was an 8 x 4 cm tender mass in the epigastrium, and roentgenograms of this area showed pancreatolithiasis Laparotomy revealed an enlarged and nodular pancreas Operative pancreatogram demonstrated an obstructed and dilated pancreatic duct Attempts at pancreatolithotomy were unsuccessful A Roux Y pancreatojejunostomy was constructed over a number 12F T tube, a limb of which was brought through a jejunostomy opening and out the abdominal wall (Fig 1)

The T tube remained in place for 45 days and drained from 78 cc to 185 cc of fluid daily (average 268 cc per day) Pancreatograms obtained on two occasions during this period showed absence of connection with the biliary system but some escape of contrast media around the T tube into the jejunum indicating that the flow through the T tube *did not* represent the total output from the pancreas The pH of the pancreatic fluid varied from 7 8 to 8 1

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*From the Departments of Surgery and Bacteriology Veterans Administration Research Hospital and Northwestern University Medical School Chicago Illinois

The antibiotics used in this study were supplied by the Eli Lilly Company and by Charles Pfizer Company

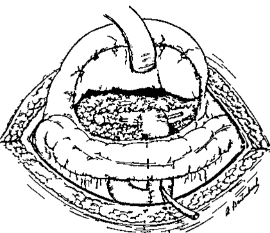


Fig 1 Roux Y pancreatojejunostomy constructed over a T tube for chronic relapsing pancreatitis

METHOD

Collections from the T tube were passed through a Seitz filter Assays f antibiotics in serum and pancreatic fluid before and after the admin tration of antibiotics were performed using the serial dilution method escribed by Price Nielsen and Welch[6] The nutrient broth used as ulture media was buffered with phosphate buffer to give a pH of 8 0 when rythromycin and streptomycin were assayed and pH 6 0 for chloram henicol as recommended by Grove and Randall[1] In addition chloram henicol was assayed using unbuffered media (pH 7 5)

Organisms isolated by routine sensitivity tests in the clinical laboratory nd sensitive to 0 5 ug/ml or less of antibiotics were selected for test rganisms in order to detect small quantities of antibiotics in pancreatic uice These organisms were more sensitive to antibiotics than those recom nended by Price and associates and by Grove and Randall for these ssays

Serum and pancreatic fluid levels were calculated by multiplying the eciprocal of the highest inhibitory dilution by the minimal inhibitory oncentration of the antibiotic against the test organisms Pancreatic fluid evels were multiplied by the volume of the sample to give the total amount f antibiotic secreted during the interval

RESULTS

Erythromycin Table 1 shows the concentration of erythromycin in the erum and pancreatic fluid before and for a 6 hour period following the ntravenous injection of 0 5 gram of erythromycin glucoheptonate dissolved n 250 ml of physiologic saline solution The injection was given over a 0 minute period and samples of blood and pancreatic fluid were col ected at 1 or 2 hour intervals after the start of the injection The total mount of erythromycin appearing in pancreatic fluid during the 6 hour eriod was 52 5 ug which is approximately 0 01% of the total dose of he drug given The highest concentration in pancreatic fluid occurred 2 ours after beginning the injection and amounted to 10% of the highest oncentration found in the serum This experiment repeated on a second ccasion using the same dose and collection periods gave essentially the ame results

Table 1 Concentration of Erythromycin in Blood and Pancreatic Fluid

SERUM			PANCREATIC FLUID			
					ERYTHROMYCIN	
TIME HOURS	INHIB DIL /	ERYTH ug/ml	SAMPLE VOLUME	INHIB DIL /	ug/ml	TOTAL ug
0†	0	0	30	0	0	0
1	640	16				
2	160	4	30	64	1 6	48
3	128	3.2				
4	64	1 6	20	8	0.2	4
5	32	0 8				
6	16	0 4	10	2	0 05	0.5

0 5 gm of erythromycin was given intravenously over a 30 minute period immediately after the control determinations The minimum inhibitory concentration of the antibiotic against the test organism was 0 025 ug/ml for all determinations

†Control

Table 2 Concentration of Streptomycin in Blood and Pancreatic Fluid

SERUM			PANCREATIC FLUID			
					STREPTOMYCIN	
TIME HOURS	INHIB DIL /	STREP ug/ml	SAMPLE VOLUME	INHIB DIL /	ug/ml	TOTAL ug
0†	0	0	40	0	0	0
1	160	16				
2	160	16	25	2	.2	5
3	128	12 8				
4	64	6 4	40	2	.2	8
5	64	6 4				
6	32	3.2	20	4	4	8
9	32	3.2	55	2	.2	11

0.5 gm streptomycin was given intramuscularly following the control determinations The minimum inhibitory concentration of the antibiotic against the test organism was 0 10 ug/ml for all determinations.

†Control

Streptomycin. One half gram of streptomycin sulphate was given intra muscularly The highest concentration in the serum was 16 *ug/ml* and was observed 1 and 2 hours after injection The highest level in pancreatic fluid, 0 4 *ug*/ml, was observed 6 hours after injection (Table 2) The *total* streptomycin excreted in a *9* hour period was 32 *ug*, approximately 0 006% of the dose injected

Table 3 Concentration of Chloramphenicol in Blood and Pancreatic Fluid

	SERUM		PANCREATIC FLUID							
				MEDIUM BUFFERED (pH 6.0)			MEDIUM UNBUFFERED (pH 7.5)			
					CHLORAMPHENICOL			CHLORAMPHENICOL		
TIME (HRS)	INHIB DIL /	CHLORAMPHENICOL ug/ml	SAMPLE VOLUME (cml)	INHIB DIL /	ug/ml	TOTAL ug	INHIB DIL /	ug/ml	TOTAL ug	
0†	0	0	16	0	0	0	0	0	0	
2	14	7								
3	20	10	40	2	1.0	40	1	0.5	20	
4	14	7								
6	6	8	48	2	1.0	48	1	0.5	20	
9	5	2.5	50	1.4	0.7	35	0	0	0	
12	<4	<2	50	1.4	0.7	35	0	0	0	
15	<3	<1.5	55	1	0.5	27.5	0	0	0	

500 mg of chloramphenicol were given by mouth following the control determinations The minimum inhibitory concentration of the antibiotic against the test organism was 0.5 ug/ml for all determinations

†Control

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EFFECTS OF GLUCAGON IN THE TOTALLY PANCREATECTOMIZED PATIENT*

FREDERICK J NEHER AND BERNARD ZIMMERMANN

In recent years total pancreatectomy is being performed with increasing frequency for pancreatic lesions which are not susceptible to the more standard procedures of radical pancreaticoduodenectomy and distal pancreatectomy [1] Although this operation is technically feasible, the consequent severe metabolic derangements constitute only a partially solved problem [2] A better understanding of these metabolic defects must be obtained if total pancreatectomy is to be more widely acceptable

A major physiologic defect in these patients results from the loss of the pancreatic islets and their endocrine secretions Insulin requirement in these patients is characteristically low, but the therapeutic dosage range compared to that of patients with spontaneous diabetes mellitus, is severely restricted A slight insulin deficit results in rapid development of acidosis and coma, while a slight excess causes severe hypoglycemia

In 1945 Thorogood and Zimmerman[3] found that the insulin requirement of alloxan diabetic dogs was markedly decreased by total pancreatectomy, but ketonuria was sharply increased and when insulin was withheld the animals rapidly developed acidosis and coma From this they deduced that the islet alpha cells, which are relatively unaffected in the alloxan diabetic dog, constituted a source of hormone which has not only a hyperglycemic effect but an antiketogenic one as well

Analogous experiments justifying a similar conclusion have recently been done on pigs Though pancreatectomized pigs develop ketosis they have only the mildest hyperglycemia and glycosuria Alloxan pigs on the contrary display severe diabetes with minimal ketosis

In 1948 Zimmermann and Donovan,[4] using a crude preparation of glucagon, demonstrated a biphasic effect on blood ketone levels in acute experiments on depancreatized dogs The recent isolation of a highly purified glucagon† preparation stimulated this study of the effect of

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†The glucagon used in this study was supplied by Dr W R Kirtley Lilly Research Laboratory (Lot #208 158B 214B)

*From the Departmer
Minnesota This work
Society Fluid Research
Grant 49G

glucagon on totally pancreatectomized humans who have no endogenous insulin or glucagon [5]

METHOD

Four patients who had previously undergone total pancreatectomy were admitted to the hospital and observed for periods up to 2 weeks during which their diet and insulin requirement were controlled The usual diet and insulin were given the day prior to the test and all food, fluid, and insulin were withheld at midnight At 8 00 a m an intravenous infusion of normal saline (control) or normal saline and glucagon was started The infusions were continued for 4 hours or 8 hours at the rate of 250 cc /hr and the glucagon content was so adjusted that the patients received 10 µg/kg /hr Blood ketone and glucose determinations were done at the start of the test and each 2 hours during the 8 hour test period [6] Urine was collected by indwelling catheter for two consecutive 4 hour periods and quantitative nitrogen and glucose and qualitative acetone determinations were done A total of 6 control and 13 glucagon tests were run on the 4 totally pancreatectomized patients which were studied

RESULTS

In all 4 patients glucagon infusion uniformly caused blood glucose levels to rise sharply during the first 4 hours while blood ketones after an initial rise during the first 2 hours levelled off or declined slightly during the second to fourth hours However, after 4 hours the blood ketone levels invariably showed a sharp rise usually reaching the levels attained by saline controls or higher values at the end of 8 hours

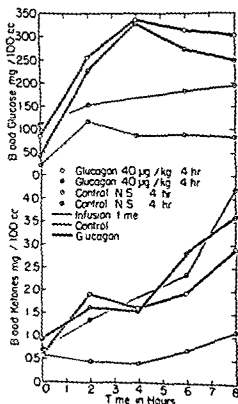


Fig 1

Figure 1 shows the results in the first patient studied. The two control infusions show a rather close relation between the course of the blood ketones and the blood glucose. During one control test the blood glucose rose gradually from 109 mg.% to 195 mg.% while the blood ketones rose steadily from 0.72 mg.% to 4.20 mg.%. The second control experiment is of interest in that the patient was hypoglycemic during the entire 8-hour period although clinical signs were minimal and apparent only in retrospect. The hypoglycemia can probably be attributed to a low caloric intake on the preceding day and the pre-study fast. During this test the blood glucose was only 22 mg.% at the start and 83 mg.% at the end of 8 hours The blood ketone level shows little rise during this period, indicating but little ketogenesis in the absence of hyperglycemia.

The two 4-hour glucagon infusions (Fig. 1) produced in the same patient a striking hyperglycemia reaching its peak at the end of the infusion and decreasing somewhat during the 4-hour post-infusion period. An initial sharp rise in blood ketone levels during the first 2 hours of glucagon infusion is followed by a slight depression during the second 2 hours, after which the level rises sharply again during the 4 post-infusion hours.

Since the above study suggested an antiketogenic effect of glucagon, three other totally pancreatectomized patients were studied using both 4-hour and 8-hour glucagon infusions. Figures 2 and 3 show that a slight depression of ketosis occurs uniformly between the second and fourth hours. However, continued administration of glucagon for 8 hours did not further

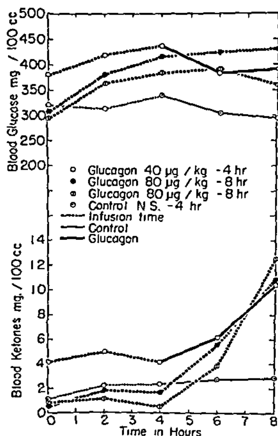


Fig. 2.

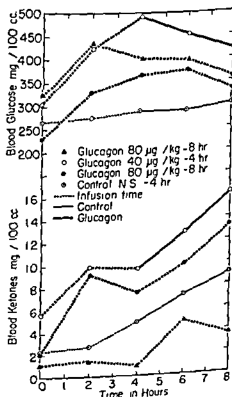


Fig. 3.

suppress ketogenesis and after 4 hours the blood ketone levels rose to approximately those of the 4 hour infusions Thus the depression in rate of ketogenesis caused by glucagon is apparently a transient phenomenon

All patients showed a marked glycosuria in response to glucagon infusion Four hour urine specimens collected during infusion usually contained 20 to 40 gm of glucose while control specimens contained at most 2 to 3 gm Nitrogen excretion is only questionably elevated in these acute experiments Because of the well documented hazard to the pancreatectomized patient of withholding insulin it was not possible to safely conduct prolonged investigations of nitrogen excretion

DISCUSSION

It has been generally accepted that depletion of liver glycogen caused by fasting or hypoinsulinism enhances ketosis Since the hyperglycemic effect of glucagon derives from conversion of liver glycogen into blood glucose[7] an explanation of these findings might be that glucagon exhibits a constant slight antiketogenic effect until the available store of liver glycogen is depleted after which ketogenesis takes place at a rapid rate The fact that the greatest rise in blood glucose in response to glucagon occurs in the first 4 hours and levels off thereafter would further indicate that after 4 hours of glucagon infusion the liver glycogen stores have become depleted following which inhibition of ketogenesis is lost

Contrary to the conventional theory Winternitz[8] and others have found that severe depletion of liver glycogen is not a prerequisite for the development of ketosis In both the alloxan diabetic rat and the diabetic human severe ketosis may coexist with substantial liver glycogen reserves This liver glycogen may of course be metabolically unavailable

No conclusive evidence was found that glucagon increased nitrogen excretion Increased nitrogen excretion would indicate accelerated protein catabolism or gluconeogenesis a finding described by Kalant[9] in fasting rats treated with glucagon

On the basis of this study it is apparent that *glucagon has only* a transient depressant effect on the development of ketosis in the patient with a total pancreatectomy so that at the present time there is no technique for incorporating it into the regimen of replacement medication However further studies are in progress to determine whether glucagon administered together with insulin might serve as a buffer to widen the safe therapeutic dosage range of insulin in these patients by countering the severe hypoglycemic effect of excess insulin

SUMMARY

1 Glucagon caused a marked hyperglycemia and a corresponding glycosuria in the totally pancreatectomized patient

2 A slight transient inhibition of ketogenesis is caused by glucagon However after 4 hours despite continued glucagon infusion an escape occurs and blood ketones rise rapidly

3 Measurements of urinary nitrogen gave suggestive evidence that glucagon was capable of stimulating gluconeogenesis from protein while inhibiting ketogenesis from fat.

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Heart

A. Myocardial Physiology in Heart Surgery

EXPERIMENTAL STUDIES ON THE CARDIAC LYMPHATICS*

PHILIP R ALLISON AND DAVID C SABISTON, JR

Although much work has been directed toward the anatomy and physiology of the arterial and venous circulation of the heart, few studies have been performed on the cardiac lymphatics Rudbeck[1] is given credit for the first description of the lymphatic vessels of the heart in 1653 In 1924 Aagaard[2] wrote a detailed monograph on the subject and summarized the work of others until that time The problem was further studied by Patek[3] in 1939 As a result of these studies the concept is now held that the heart possesses a diffuse network of lymphatics and is in a sense a "lymphatic sponge".

It appears reasonable to believe that the lymphatics of the heart play an important role in the normal and pathologic physiology of the heart This, however, is at present poorly understood and lacks confirming experimental evidence The observations to be discussed in the present communication represent the initial phase of a group of studies concerning the cardiac lymphatics and consist of a demonstration of the anatomy of the sub epicardial lymphatics together with their collecting channels and lymph nodes in the mediastinum

METHOD

Twenty adult mongrel dogs were used Anesthesia was induced with intravenous nembutal (25 mg /kg) and respiration was maintained through an endotracheal tube attached to a mechanical respirator The animal was placed in the supine position and the chest was entered either through a bilateral thoracotomy in the fourth intercostal space or preferably through a median sternotomy The pericardium was opened and the edges were loosely sutured to the chest wall in order to form a cradle for the heart An injection of the cardiac lymphatics was then made by carefully placing a short No 25 hypodermic needle immediately beneath the epicardium The needle was attached by a segment of polyethylene tubing to a pressure reservoir of dye (Berlin blue or colloidal graphite) This dye was injected at a pressure of 90 to 100 mm Hg The sub-epicardial vessels of both ventricles were injected and the mediastinal lymphatics were allowed to fill The animal was then sacrificed by bleeding and the heart and mediastinal structures were removed The specimen was then placed in formalin Following fixation the lymphatic channels were dissected and diagrams made

*From The Department of Surgery The Johns Hopkins University School of Medicine and Hospital Baltimore Aided by a grant from the United States Public Health Service National Heart Institute (H 2864) and The Howard Hughes Medical Institute

RESULTS

With the use of the technique as outlined it was possible to obtain good filling of both the sub-epicardial and mediastinal lymphatics including their corresponding lymph nodes. The sub-epicardial lymphatics were extremely numerous and diffuse. These lymphatics were small and drained into larger ones which coursed along the primary branches of the coronary vessels to enter a large channel passing through the pericardial reflections with the mediastinum. It was noted that dye appeared in the mediastinum as quickly as 2 minutes after its injection into the sub-epicardial lymphatics. In the majority of specimens the anatomical pattern was similar although numerous variations were found in the mediastinal channels and in their corresponding lymph nodes. In the most typical specimens the sub-epicardial lymphatics of the anterolateral surface of the left ventricle joined at the base of the pulmonary artery and passed beneath this vessel. From this point the channel passed anterior to the right branch of the pulmonary artery and ascended on the anterior surface of the trachea to a node at the junction of the brachiocephalic artery and the superior vena cava (Fig. 1 A). The lymphatics of the anterior right ventricle usually united into a channel in the right atrio-ventricular groove and passed anterior to the ascending aorta and continued superiorly to enter a lymph node along the left subclavian artery (Fig. 1 A). On the posterior aspect of the heart the

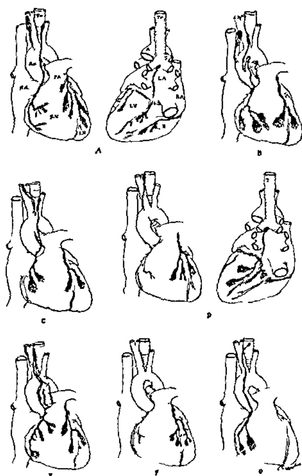


Fig. 1. Diagrams illustrating anatomical pathways of the cardiac lymphatics.

A. Anterior and posterior ventricular surfaces showing most common pattern of lymphatics.

B. Injection of right and left ventricle illustrating tortuosity of mediastinal lymphatics.

C. Diagrammatic illustration of communications in the mediastinum between the lymphatic channels from the right and left ventricles.

D. Injection of left ventricular lymphatics (anterior and posterior surfaces).

E, F, G. Diagrams of variations in the mediastinal pathways of the cardiac lymphatics.

vessels merged in the atrio ventricular groove and passed over the posterior aspect of the left atrium to lymph nodes on the posterior surface of the trachea and main bronchi (Fig 1 A)

Many variations in the anatomic pattern were found and illustrative examples are shown in Figures 1 B C D E F and G

DISCUSSION

Our interest in the problem of the cardiac lymphatics arose from two considerations first the absorption of fluid in pericarditis and secondly in association with observations in coronary arterial insufficiency The unequal clinical results of the various operations for cardiac ischemia and the certain small success that seemed to be common to all such operations raised the possibility that the lymphatics of heart might play a role in this process Further in mitral stenosis an enormous dilatation of the lymphatic vessels beneath the visceral pleura around the lung root and along the internal mammary near the phrenic nerve is evidence of the role the lymphatic system plays in this disease This apparent relief to the pulmonary circulation has been mainly overlooked or ignored because of the trivial amount of lymph flow which has been reported from the lungs of normal dogs

Drinker and others maintain the view that the cardiac lymphatics unite at the base of the heart in a single trunk which permits the collection of all the cardiac lymph by cannulation of an isolated vessel[4] Physiologic studies have been performed on the total cardiac lymph collected by this method The results of the present study demonstrate that multiple lymphatic channels lead from the heart into the mediastinum and the concept of a single cardiac lymphatic trunk appears to be an oversimplification Collection of the total lymph drainage of the heart necessitates knowledge of these pathways and cannulation of each of the lymphatics leading from the heart

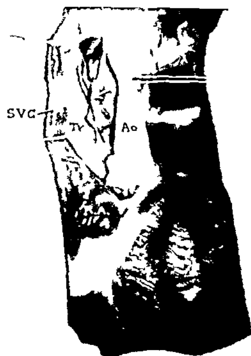


Fig 2 Photograph illustrating multiple mediastinal lymphatics filled from injection of the subepicardial lymphatics of the left ventricle with colloidal graphite

The multiple lymphatic channels in the mediastinum of an injected specimen are shown in Figure 2

In the usual pattern two or more main lymphatic trunks are formed at the anterior base of the heart and pass into the mediastinum The anterolateral aspect of the left ventricle drains largely into a channel which passes deep to the pulmonary artery and superficial to its right branch terminating in a lymph node between the superior vena cava and brachiocephalic artery The channel for the corresponding area of the right ventricle passes anterior to the root of the aorta and through the reflection of the pericardium to a node along the left subclavian artery The lymphatics of the posterior surface of the ventricles drain mainly into channels passing on the posterior aspect of the left atrium to lymph nodes on the posterior surfaces of the trachea and main bronchi However, dissection of the injected specimens emphasizes the fact that significant variations in this anatomic pattern occur

SUMMARY

1 The cardiac lymphatics of the dog have been studied by the subepicardial injection of dye in the beating heart

2 The mediastinal pathways of the lymphatics draining the heart have been determined Although a more or less consistent pattern is present in many hearts, emphasis is placed upon the variations which may occur

3 The variations in the anatomical pattern are of importance in physiologic studies involving the total collection of cardiac lymph

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SURGICAL ANATOMY OF THE CARDIAC SEPTA*

JORGE A RODRIGUEZ AND JESSE L WOFFORD

A thorough knowledge of the anatomy of the cardiac septa is of paramount importance to the surgeon Classical descriptions though correct for the most part, have dealt with the heart in a vertical plane removed from its usual anatomical site Our descriptions are based upon the heart *in situ* preserving many landmarks which are invaluable in surgical orientation

*From the Department of Surgery University of Mississippi Medical Center Jackson
Supported in part by a grant from the Mississippi Heart Association

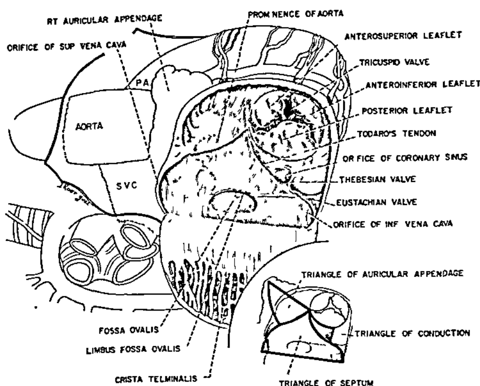

SURGICAL ANATOMY OF THE INTERATRIAL SEPTUM
RT AURICULAR APPENDAGE
PROMINENCE OF AORTA
ORIFICE OF SUP VENA CAVA
ANTEROSUPERIOR LEAFLET
TRICUSPID VALVE
PA
ANTEROINFERIOR LEAFLET
POSTERIOR LEAFLET
AORTA
TODARO'S TENDON
ORIFICE OF CORONARY SINUS
THEBESIAN VALVE
SVC
EUSTACHIAN VALVE
ORIFICE OF INF VENA CAVA
TRIANGLE OF AURICULAR APPENDAGE
TRIANGLE OF CONDUCTION
FOSSA OVALIS
LIMBUS FOSSA OVALIS
CRISTA TELMINALIS
TRIANGLE OF SEPTUM

Fig 1.

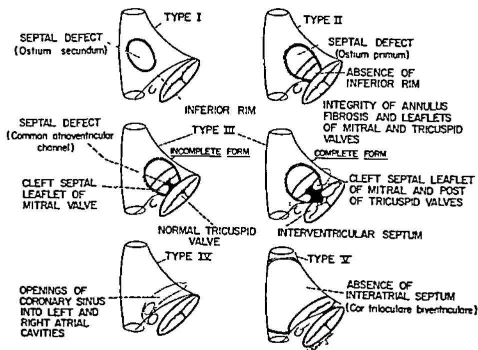

INTERATRIAL COMMUNICATIONS
TYPE I
SEPTAL DEFECT
(Ostium secundum)
TYPE II
SEPTAL DEFECT
(Ostium primum)
ABSENCE OF
INFERIOR RIM
INFERIOR RIM
INTEGRITY OF ANNULUS
FIBROSIS AND LEAFLETS
OF MITRAL AND TRICUSPID
VALVES
SEPTAL DEFECT
(Common atrioventricular
channel)
TYPE III
INCOMPLETE FORM
COMPLETE FORM
CLEFT SEPTAL
LEAFLET OF
MITRAL VALVE
CLEFT SEPTAL LEAFLET
OF MITRAL AND POST
OF TRICUSPID VALVES
NORMAL TRICUSPID
VALVE
INTERVENTRICULAR SEPTUM
TYPE IV
TYPE V
OPENINGS OF
CORONARY SINUS
INTO LEFT AND
RIGHT ATRIAL
CAVITIES
ABSENCE OF
INTERATRIAL SEPTUM
(Cor triloculare biventriculare)

Fig 2

Atrial Septum The drawing in Figure 1 shows the atrial septum It is posterior in position and not medial as described classically When viewed through the right atrial cavity, the thin wall of the septum is triangular in shape Within this triangle and midway between the caval openings the transparent fossa ovalis forms a noticeable depression in the septal wall The somewhat thickened medial rim of the fossa ovalis is continuous with the vestigial fold of the eustachian valve

A second triangular area of the septum though smaller in size is also a valuable anatomical landmark The atrioventricular node and the orifice of the coronary sinus are located within this triangle which lies close to the tricuspid valve

Interatrial Communications Abnormal openings in the interatrial septum vary and classifications to date are indistinct and not surgically oriented The following classification would seem simple and practical These types are shown in Figure 2

Surgical Implications There are a number of surgical considerations of anatomical origin which should be mentioned here

1 When a septal defect is located near the opening of the inferior vena cava one must carefully examine the entire rim of the defect in order to distinguish it from a well developed eustachian valve If the free edge of this valve should be sutured by mistake to the rim of the defect it would result in an improper transplantation of the inferior vena cava to the left atrium

2 In defects located near the elevation of the ascending aorta when the medial rim is absent difficulty in correction increases as well as the danger of lacerating the wall of this vessel

3 In suturing an atrial septal defect one may produce cardiac arrhythmias This may be due to the stimulation of the atrioventricular node located near the tricuspid valve In such a case the needle should be withdrawn and reinserted in a different place

Ventricular Septum For the purpose of description the interventricular septum may be divided into an infundibular portion a ventricular portion and a membranous portion The *infundibular* portion is limited by the pulmonary valve at its superior end while the crista supraventricularis the base of the papillary muscle of the conus and the origin of the moderator band form the boundaries toward the ventricular cavity The lateral limits are indistinct The right lateral cusp of the aortic valve is very close to the septal wall at the site of the origin of the crista supraventricularis The *ventricular* portion of the septum extends from the inferior edge of the infundibular portion to the apex of the heart The tricuspid valve limits its posterior boundary The *membranous* portion of the septum is within the depths of the subinfundibular fossa hidden by the crista supraventricularis and the tricuspid valve It is a small transparent triangular area approximately 8 to 15 mm in diameter This space is found between the right lateral and posterior cusps of the aortic valve and a muscular rim of the ventricular septum It is in extremely close relationship to the tricuspid valve and the bifurcation of the bundle of His

Interventricular Septal Defects We present a simplified classification of interventricular septal defects as illustrated in Figure 3

Surgical Implications (1) The bundle of His bifurcates at the membra

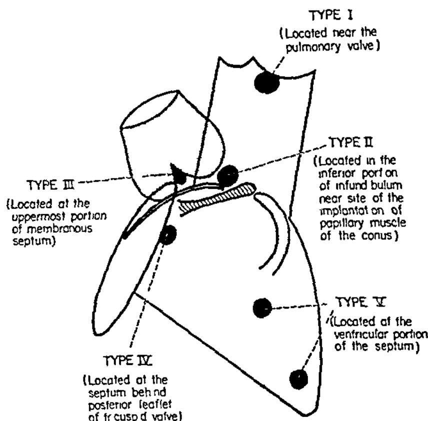


Fig 3

nous portion of the interventricular septum For this reason Type II and III defects located here may be difficult to close without stimulation or injury to the conduction system of the heart Should cardiac arrhythmias occur at the time of closure it may be necessary to withdraw the needle and reinsert it at a different point However in induced cardiac arrest it would seem difficult to detect injuries of the conduction system This emphasizes the need for a thorough understanding of these anatomical relationships (2) In cases of Type III defects the rim may be partly formed by the crista supraventricularis One must remember that this muscle is close to the aortic valve A laceration or distortion of the right lateral cusp of the aortic valve may be produced by deep insertion of a suture at this point

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THE DISTRIBUTION OF THE OCCLUSIVE PROCESS IN CORONARY ARTERIOSCLEROSIS A POSTMORTEM ROENTGENOLOGIC STUDY*

RICHARD T McDONALD, D EMERICK SZILAGYI, LLOYD C FRANCE AND JOSEPH C SIERACKI

For the evaluation of the applicability of direct techniques (endarteriectomy and/or grafting) in the surgical management of coronary arterial occlusive disease, the knowledge of the type of distribution of the occlusive process is of great importance The excellent studies[1 2] published on the pathologic anatomy of coronary atherosclerosis clarified many aspects of the disease but were not undertaken to explore the problem of operability To amplify the information now available from the point of view of the feasibility of direct surgical attack on occlusive coronary disease, we have during the last 12 months examined 113 hearts obtained at autopsy.

METHOD

In obtaining the material to be studied our initial plan called for the examination of a consecutive series of hearts, the only factors of selection were those of practical exigencies In this phase of the study 69 of 102 hearts were available for investigation This group contained a high proportion of patients (38%) with no clinical symptoms and with normal angiographic examination and whose ages ranged from 8 to 79 years In the next phase of the study, investigation was limited to hearts from patients over 50 years of age or with clinical arteriosclerotic heart disease regardless of age The hearts were usually studied immediately after death and never more than 24 hours later If the examination was delayed the hearts were refrigerated at 35 to 40°F

For the demonstration of the coronary artery system an injection method as described by Meade and Bledsoe[3] was used with the exception that the barium mixture contained 1/6 glycerine by volume and radiograms were made in two planes after rotation of 180° Every specimen was then serially sectioned and the angiographic and pathologic findings carefully correlated

RESULTS

Early in the study it became evident that films in two planes were adequate to demonstrate even minor alterations in the arterial walls Branches, however, could not be evaluated if less than 0 5 mm in diameter Failure to demonstrate occlusions angiographically also occurred in small inconstant branches when there was no retrograde filling Since these had no practical surgical importance they were left out of final evaluation Complete failure of the constant branches to fill was recognized by reference to the normal anatomical pattern † Since the stitch ligature

†An exception to this statement is discussed in connection with the circumflex branch

*From the Department of General Surgery and Laboratories Henry Ford Hospital Detroit Supported in part by a grant from the Michigan Heart Association With the assistance of Dr W R Eyler Roentgenologist in Chief Henry Ford Hospital in obtaining the coronary angiograms

tended to narrow the ostia, changes in their diameter were evaluated grossly rather than roentgenologically The three main coronary arteries were assessed and classified according to the following pathological changes tortuosity, calcification stenosis (singular or multiple) or occlusion

In the total group of 113 specimens 39 were angiographically and grossly normal Nineteen males and 20 females, aged 8 to 79 years, comprised this group The study of these specimens afforded us information with respect to the normal appearance of intact hearts as demonstrated by this method The posterior septum was supplied by the right coronary artery in 31 cases (80%), and by the left coronary artery in 5 cases (13%) The remaining 3 cases (7%) were also of predominant right coronary artery pattern but in addition the circumflex artery was very small The deficiency of the circumflex artery was compensated for by unusually large left obtuse branches The importance of this will be discussed later

Seventy four of the 113 injected specimens demonstrated lesions of one or more coronary arteries The specimens were divided into 4 groups, namely those with (1) no subjective evidence of coronary disease, (2) clinical symptoms other than angina pectoris with positive electrocardiographic findings, (3) angina pectoris, and (4) clinical symptoms in association with diabetes mellitus (Table 2)

In the first group 31 patients demonstrated no clinical symptoms or laboratory findings Nine females with an average age of 62 years and 22 males with an average age of 61 5 years comprised this group Three patients had one artery disease Eight had two artery disease and 20 patients had three artery disease In all, the lesions consisted of 26 tortuosities, 6 calcifications 15 single stenoses 8 multiple stenoses, and 9 occlusions

Among the patients who had manifest symptoms of arteriosclerotic heart disease without angina pectoris there were 8 females and 14 males, with an average age of 65 years One coronary artery was involved in 4 instances, 2 arteries in 8 instances and all three arteries in 10 specimens A further analysis showed that the lesions consisted of tortuosity in 3, calcification in 4, single stenosis in 15 multiple stenoses in 16, and occlusion in 16 cases

Of the 12 patients who exhibited angina pectoris, 11 were males with an average of 69 9 years, 1 was a female, aged 54 years In 8 specimens 3 arteries were involved and in the remaining 4 specimens 2 arteries were involved Valvular lesion was present in only 1 specimen and this consisted of aortic stenosis The average duration of the angina was 1 86 years Nine patients had had previous myocardial infarctions The types of lesion were calcification (4), single stenosis (6) multiple stenosis (12) and occlusions (9)

The group with diabetes mellitus numbered 9 and included 2 patients with angina pectoris The average duration of the diabetes, varying in degree from mild to severe, was 9 6 years Four females with an average age of 60 2 years and 5 males with an average age of 65 0 years comprised the group In 8 specimens all 3 arteries were involved and in the 4 remaining specimens 2 artery disease was present The lesions consisted of 1 instance of calcification, 1 instance of single stenosis, 10 instances of multiple stenosis and 14 instances of occlusion

DISCUSSION

Two plane radiograms of the injected hearts proved quite adequate to visualize lesions in the larger constant appearing coronaries The only area where difficulty in interpretation arose was in the region of the circumflex coronary artery In 6% of the normal hearts a small circumflex artery was present This was at times so minute that serious doubt arose as to whether it was actually occluded The presence of a large obtuse branch is a good indication that the small size of the circumflex artery is caused by anatomical rather than pathological factors The potential importance of the small circumflex artery in clinical coronary angiograms — when these are available — is self-evident.

Since the left main coronary artery was so short as to be entirely taken up by the polyethylene catheters it usually did not cast an angiographic image capable of interpretation For this reason only the right, left anterior descending, and circumflex arteries are given individual consideration Lesions which occurred in the left main coronary artery are included among those listed for the left anterior descending artery

In the hearts with asymptomatic coronary artery disease 32 arteries were sclerotic or tortuous Since the lumen remains unobstructed, calcification of the arterial walls does not seriously interfere with luminal blood flow and with the myocardial blood supply On the other hand, marked tortuosity impairs volume flow, however, owing to the large safety margin in peripheral resistance in the coronary system, the reduction is not evident clinically [4]

Operability. With a clinical experience of over 250 peripheral grafting procedures for technical guidance and with the experimental observations of 70 operations on the canine coronary arterial system, we have attempted to establish criteria of operability for the occlusive lesions described in this study Obviously these criteria must be arbitrary No one can state, categorically, the lower limit in the dimensions of an artery permitting direct surgical attack It is likewise a matter of speculation how much benefit one may reasonably expect from the removal of an occlusive lesion in any given heart, which is usually the seat of a complex of pathological lesions It must also be stressed that in setting up these criteria we do not infer that they represent operability in a practical clinical sense These criteria are rather theoretical guides based upon anatomical appearances in a lifeless specimen, we merely assume that on the grounds of these anatomical facts the blood flow through the coronary system in question could be improved

On these grounds, we regarded as accessible to direct surgical attack those occlusive lesions that are located within 5 cm of the origin of the 3 coronary arteries, provided, however, the luminal diameter of the artery at this level is no less than 2 mm and a satisfactory outflow tract is present Since quite frequently a remediable lesion in one coronary artery is found in association with other occlusive lesions which are not subject to surgical correction, the hearts that lend themselves to direct surgical therapy must be further subdivided into those in which the blood flow may be improved as opposed to those in which since the occlusive lesions are completely remediable, the blood flow can be returned to normal The incidence of lesions

remediable only in the sense of palliation and of lesions remediable to the extent of cure is given in Table 2

Table 1. Distribution of Occlusive Lesions

	NON CLINICAL			CLINICAL			ANGINA			DIABETES		
	R	AD	C	R	AD	C	R	AD	C	R	AD	C
Single Stenosis	4	9	2	4	6	5	2	..	4	..	..	1
Multiple Stenoses	2	3	3	5	8	3	3	6	3	4	3	3
Single Occlusion	2	5	2	8	6	2	7	4		5	5	4
TOTAL	8	17	7	17	20	10	12	10	7	9	8	8

R = Right coronary artery
AD = Left main and left anterior descending coronary arteries
C = Circumflex coronary artery

Table 2. Estimate of Operability

PATIENTS CLINICAL CLASSIFICATION	NO OF ARTERIES INVOLVED	INOPERABLE NO OF CASES	PALLIATION (POSSIBLE) NO OF CASES	CURE (POSSIBLE) NO OF CASES
No clinical	1	5	1	5
	2	2	6	–
A.S H D	3		2	–
Clinical	1	2		–
	2	1	4	3
A.S H.D	3	3	6	–
Clinical	1	..	..	..
A H.S.D	2	2	4	..
with	3	2	4	..
Angina				
Clinical	1		–	–
A.S H.D	2		1	–
with	3	5	3	–
Diabetes				
TOTAL		22 (35.9%)	31 (52 4%)	8 (12 7%)

SUMMARY AND CONCLUSIONS

One hundred and thirteen unselected hearts (39 grossly normal and 74 with one or more lesions of the coronary system) were examined roentgenologically and pathologically at autopsy

1 Forty three percent of the pathological specimens had significant coronary occlusive disease which had not been manifest clinically That these were indeed early lesions is borne out by the finding that the majority of lesions involved only one coronary artery and most were theoretically curable

2 Seventy three percent of the cases without angina but with other clinical symptoms had involvement of two or more arteries however only 10% of these lesions were theoretically curable

3 Angina did not occur unless at least two arteries had extensive involvement None of the patients with angina had curable lesions All but one had had previous myocardial infarctions the latter suffered infarction terminally All were cardiac deaths Angina pectoris instead of being a symptom of early involvement in this series represented severe derangement of the coronary arteries

4 The number of patients with diabetes was too small to draw any conclusion However their coronary disease tended to be more severe and more widespread None of these patients had theoretically curable disease

5 In general coronary arteriosclerosis when clinically manifest tends to be generalized In this series 35 9% of the specimens were inoperable in 52 4% of the cases palliation could probably have been achieved and only 12 7% of the cases had theoretically curable lesions

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TENSILE STRENGTH OF MYOCARDIUM*

Ernst Beck and E J Beattie Jr

The low tensile strength of sutured muscle is well known to all surgeons The development of intracardiac surgery necessitates the suturing of myo cardium From personal experience we know that the suturing of myo cardium can be dangerous This study was undertaken to learn the strength of myocardium sutured by various techniques

METHOD

Thirty one normal dog hearts were used Twenty six hearts were from freshly autopsied animals In 5 animals studied the living myocardium was sutured during an experiment with the heart lung pump apparatus In the living animals the sutures were left in place for from 6 hours to 8 days before testing the tensile strength The hearts varied in weight from 80 to 280 gm In the autopsied hearts a standard incision 4 cm long was made into the left ventricle parallel to the anterior descendant branch of the left coronary artery The ventricular wall varied in thickness from 0 8 to 2 0 cm In the living heart the incision ranged from 2 5 to 6 cm in length In 4 living hearts the incision was made in the right ventricle in the 5th heart the incision was in the left ventricle

All hearts were sutured with silk In the dead hearts all sutures were 1 0 cm apart and 0 4 cm back from the edge of the incision The sutures were either continuous or interrupted The sutures were placed either through the full thickness of the myocardium (4 0 silk) or superficially 0 5 cm deep in the myocardium (3 0 silk)

In sutures reinforced with a graft a 3 x 6 cm piece of pericardium was used In the living hearts continuous through and through sutures were used varying in number from 6 to 20

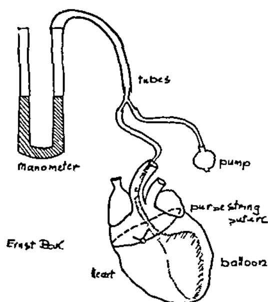


Fig 1

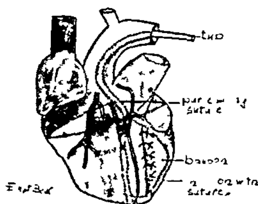


Fig 2

A thin walled rubber balloon attached by rubber tubing (See Fig 1) to a mercury manometer was inserted into the ventricular cavity through the mitral valve in those cases with the left ventricular incision, and through the tricuspid valve in the case with the incision in the right ventricle The tubing was fixed in place by a purse string suture closing the mitral or tricuspid valve The strength of the suture line was tested either by inflating the balloon to the pressure at which the anastomosis

Table 1

NUMBER OF DOGS	DEAD OR LIVING	HEART WEIGHT	MYOCARDIAL THICKNESS	RUPTURING PRESSURE IN MM OF Hg
Group 1 Interrupted superficial sutures with continuous balloon pressure Three sutures of 3 0 silk				
3	D	270	1 7	320
		185	1.5	300
		70	1 7	280
Group 2 Continuous superficial sutures with continuous balloon pressure Five sutures of 3 0 silk				
4	D	110	1 0	400
		60	0 8	360
		100	0 8	360
		75	1 4	400
Group 3 Interrupted through and through sutures with continuous balloon pressure Three sutures of 4 0 silk				
5	D	280	2 0	500
		150	1 0	300
		150	1 5	300
		110	2 0	400
		230	2 0	400
Group 4 Continuous through and through sutures with continuous balloon pressures Five sutures of 4 0 silk				
2	D	130	1.2	400
		110	1 2	440
Group 5 Continuous through and through sutures with pericardial graft Continuous balloon pressure Five sutures 4 0 silk				
6	D	150	1.5	450
		160	2 0	600
		150	2 0	620

ruptured or by inflating the balloon to 300 mm Hg and reducing the pressure to 0 The number of times the pressure could be raised without separation of the suture line was recorded In every case the suture material remained intact and the rupture occurred when the sutures were pulled out of the myocardium

RESULTS

Group 1. In autopsied hearts 3 interrupted 3 0 silk sutures were placed ½ cm deep in the myocardium The continuous pressures required to separate the suture lines were 320, 300 and 280 mm Hg respectively

Group 2. In 4 autopsied hearts the left ventricle was closed with a continuous suture line (3 0 silk) placing each suture 1 cm apart and 0 5 cm deep The continuous pressures required to separate the suture lines were 400, 360, 360 and 400 mm Hg respectively

Group 3. Five autopsied hearts were used in this group The left ventricle was closed with 3 interrupted through and through sutures of 4 0

Table 2

NUMBER OF DOGS	DEAD OR LIVING	HEART WEIGHT	MYOCARDIAL THICKNESS	RUPTURING PRESSURE IN MM OF Hg
Group 5 (continued)				
		100	0 9	500
		110	1 6	520
		105	1.2	600
Group 6 — Continuous through and through suture of the right ventricle continuous balloon pressure 6 12 20 sutures of 3 0 silk				
3	L	100	0 7	420
		80	0 6	440
		150	0 7	360
Group 7 — Continuous sutures with interrupted balloon pressure 5 5 5 5 17 and 12 sutures of 3 0 silk				
6	D	110	1.5	300x8
	D	120	1.5	300x75
	D	110	1.5	300x26
	L	150	1 0	300x16
	L	150	1 0	300x17
	L	90	0 7(right ventricle)	300x75
Group 8 — Continuous through and through sutures with pericardial graft Interrupted balloon pressure Five sutures 3-0 silk				
2	D	170	1.2	300x200 no rupture
		70	1 4	300x200 no rupture

silk The pressures required to separate the suture lines were 500 300 300, 400 and 400 mm Hg respectively

Group 4. In 2 autopsied hearts, the standard 4 cm incision was closed with continuous through and through 4 0 silk sutures placed 1 cm apart The pressures required to cause separation of the suture lines were 400 and 440 mm Hg

Group 5. Six hearts from autopsied dogs were used The standard 4 cm incision was closed with through and through continuous silk sutures of 4 0 silk using a pericardial graft reinforcement The pressures required to separate the suture lines were 540, 600, 620, 500, 520 and 600 mm Hg respectively

Group 6. In this group were 3 living dogs Incisions of 2 5, 4 0 and 6 cm were made The incisions were closed with continuous through and through sutures using 3 0 silk and, respectively 6, 12, and 20 sutures The dogs were autopsied respectively 8 days, 8 days and 6 hours after operation The required pressures to separate the suture line 8 days later were 420 and 440 mm Hg The 6 hours old suture line required only 360 mm Hg

Group 7. Four autopsied dogs were used The standard incision was closed with superficial continuous sutures in one case and through and through continuous sutures in 3 other cases Interrupted pressure was used In the continuous superficial suture line, separation occurred when the pressure was raised to 300 mm Hg 8 times In the continuous through and through suture lines, separation occurred after the pressure had been raised to the same figure 75, 26 and 16 times, respectively A 5th dog in this group was alive at the time the sutures were placed A 6 cm incision was closed with 16 through and through continuous sutures using 3 0 silk Nine hours after operation the dog was autopsied The suture line separated after the pressure had been raised to 300 mm Hg 17 times A 6th living heart had a 1 x 1 cm defect made in the right ventricle which was closed with 12 through and through silk sutures When the heart was studied eight days later the pressure was raised to 300 mm Hg 75 times before separation of the suture line occurred

Group 8. A standard incision was closed with through and through continous sutures reinforced with pericardial graft in 2 autopsied hearts Raising the pressure to 300 mm Hg 200 times failed to cause separation of the suture line

SUMMARY AND CONCLUSIONS

1 This study was undertaken to determine the effect of the suture in the strength of a myocardial incision in the dog

2 Twenty six autopsied hearts were used and 5 living dog hearts were used

3 The incisions were sutured with interrupted or continuous silk either through all or a superficial part of the myocardium A balloon was placed in the ventricular cavity and inflated to various pressures

4 The superficial interrupted suture lines separated at an average pressure of 300 mm Hg The continous superficial sutures withstood a pressure of 380 mm Hg The interrupted through and through sutures withstood an average of 380 mm Hg pressure The continuous sutures through all the thickness of the myocardial wall withstood an average of 420 mm Hg

The continuous through and through sutures using a pericardial graft withstood an average of 560 mm Hg

5 Continuous suture lines through all thickness of the myocardial wall using a pericardial graft were unbreakable using alternating pressures to 300 mm Hg elevated 200 times

6 The strength of the sutured myocardium immediately after being sutured seems greater in the thicker myocardium

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CARDIAC METABOLISM UNDER CONDITIONS ASSOCIATED WITH OPEN HEART OPERATIONS

I CORONARY SINUS FLOW AND MYOCARDIAL OXYGEN CONSUMPTION*

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We have measured coronary sinus flow and myocardial oxygen consumption of the canine heart in its usual working state under anesthesia and have compared this to the coronary sinus flow and myocardial oxygen consumption under conditions of cardiac inflow occlusion ventricular fibrillation and cardiac arrest due to acetylcholine or potassium citrate

METHOD

Mongrel dogs weighing 10 to 16 kg were anesthetized with thiopental intravenously After the thorax had been opened by an incision through the fourth intercostal space ventilation was maintained by a device providing intermittent positive pressure The azygos vein was ligated at its entry into the superior vena cava Both cavae were encircled by umbilical tapes for subsequent inflow occlusion A plastic catheter was inserted into the superior vena cava by way of the right jugular vein and into the inferior vena cava by way of the right femoral vein These provided pull off to the Model III DeWall pump oxygenator Return from the oxygenator was pumped into the aorta through the right carotid artery Systemic pressure was continuously recorded by means of a catheter in the right femoral artery A continuous record of the ECG was also made

A balloon catheter 12F or 14F was then passed through the right atrium into the coronary sinus The coronary sinus ostium was tightened by means of an externally placed circumferential suture and the balloon was inflated The end of the catheter was kept at a level 10 cm below the atrium to provide a constant siphon effect Coronary sinus drainage was measured

*From the Department of Surgery St Louis University School of Medicine Supported by a grant from the Douglass Foundation and Grant H 2859 from the National Heart Institute of the National Institutes of Health United States Public Health Service

either directly or by an electromagnetic flowmeter and returned to the venous side of the pump oxygenator Oxygen saturation was measured photoelectrically Arterial oxygen saturations were determined on samples drawn through the catheter in the femoral artery

Determinations were made over a period of several minutes with the coronary sinus loss being replaced by simultaneous infusion of an equivalent amount of blood The pump oxygenator was started and inflow to the heart was occluded Repeated determinations were made in each animal under conditions of inflow stasis Further determinations were then made with either (a) ventricular fibrillation induced by a 5 to 10 volt shock (b) cardiac arrest resulting from 1% acetylcholine injected into the root of the aorta (c) cardiac arrest following injection of potassium citrate into the aortic root

Calculations of oxygen consumption were made using actual heart weights at autopsy Oxygen extraction is expressed as percentage of oxygen reduction from artery to vein

RESULTS

Satisfactory data were obtained from 18 animals The control determinations made before cardiopulmonary bypass varied considerably from animal to animal This is consistent with reports by previous workers[1,5,6] The average figures for coronary sinus flow and myocardial oxygen consumption of the normally beating heart under anesthesia were slightly lower than reported values determined by indirect methods[1,2]

Because of the variations in the control values from animal to animal average values were not used as a basis for comparing the test conditions but rather the determinations made during a test condition were compared to those of the control period of the same animal This we feel lends more significance to the comparison A graphic representation of the changes in coronary sinus flow under various conditions is shown in Figure 1 Figure 2 is a similar representation of myocardial oxygen consumption The variations in oxygen extraction are presented in Figure 3

Inflow Occlusion Relieving the heart of the major *portion* of its work load by placing the animal on the pump oxygenator and occluding return to the heart was associated in all 18 animals with a reduction in oxygen consumption the average reduction being 35% Coronary flow was dependent upon perfusion rate and blood pressure and was generally reduced below the level observed during the control period Oxygen extraction amounted to 40% as compared to 55% for the control period

Ventricular Fibrillation In the 5 animals with ventricular fibrillation oxygen consumption was reduced by 40% The coronary sinus flow was decreased in some animals increased in others with the average of all determinations being 40% greater than flow during the control period Oxygen extraction was 25%

Cardiac Arrest In 4 animals cardiac arrest with acetylcholine was associated with a reduction in oxygen consumption of 75% over the control period In 8 animals arrest with potassium citrate resulted in a 95% reduction In all 12 animals the coronary sinus flow was greatly increased and oxygen extraction decreased

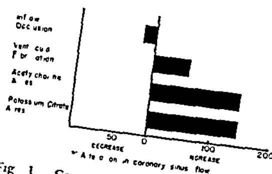


Fig 1 Coronary sinus flow in various states expressed as a percentage of loss or gain over control values

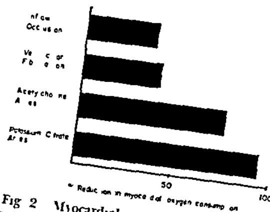


Fig 2 Myocardial oxygen consumption in various states expressed as a percentage change from control values



Fig 3 Myocardial oxygen extraction expressed as percentage reduction of oxygen saturation from artery to vein

DISCUSSION

The many variables inherent in studies of coronary sinus flow and calculations of oxygen consumption per unit of tissue are recognized. The use of actual heart weights, which in this series of dogs were at considerable variance with the averages of Herrmann,[3] and the maintenance of a constant siphon effect on the coronary sinus, permits accuracy greater than in many indirect methods

There is considerable evidence that myocardial oxygen consumption is more a function of blood pressure than of cardiac output[4] During the periods of cardiopulmonary substitution, blood pressures were generally 40% lower than during the control periods, while cardiac output was negligible There was not enough variation in blood pressures, however, to permit analysis of oxygen consumption in relation to this factor

In the test conditions employing acetylcholine, there remained occasional bizarre myocardial contractions, while with potassium citrate there was complete arrest

It was observed in most instances that myocardial oxygen consumption following reinstitution of cardiopulmonary function was lower than the pretest value Among the many inferences which may be drawn from these observations is the possibility that myocardial metabolic processes are so altered by the test conditions that the heart becomes unable to utilize oxygen to the extent that it previously did It is in fact possible that the oxygen consumption measured during the test conditions does not reflect oxygen requirement of the heart, but rather, limited ability to utilize oxygen Further study of this is in progress

SUMMARY

Coronary sinus flow and oxygen consumption of the canine heart have been measured during inflow occlusion ventricular fibrillation and arrest due to acetylcholine or potassium citrate These have been compared to values determined in the same anesthetized animals prior to establishment of these test conditions

Coronary sinus flow was decreased during inflow occlusion alone but *increased during ventricular fibrillation and cardiac arrest*

Myocardial oxygen consumption was decreased 35% during inflow occlusion 40% during ventricular fibrillation 75% with acetylcholine arrest and 95% with potassium citrate arrest

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CARBOHYDRATE METABOLISM OF THE ISOLATED PERFUSED DOG HEART*

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ROY R VETTO AND K ALVIN MERENDINO

The literature contains many apparently accurate appraisals of the gas and carbohydrate utilizations by the myocardium of intact animals and man[1 2 3 4 5 6 7 8 9] There is no unity of evidence however regarding the kind and degree of metabolic activity of the semi isolated heart that is the beating or arrested non working heart during complete heart lung bypass

Induced cardiac arrest has been shown experimentally to be sound Clinical experience is increasing daily However there is little under

*From the Department of Surgery Uni[illegible] f Med cine Seattle
Aided by Public Health Service Grant [illegible] Initiative 171
Funds from the State of Washington for

standing of the physiological consequences of induced cardioplegia, particularly as it relates to the energy metabolism of the arrested myocardium Our interest in this area led us to devise an experimental preparation which would allow a precise, controlled study of the isolated mammalian heart during perfusion and induced arrest

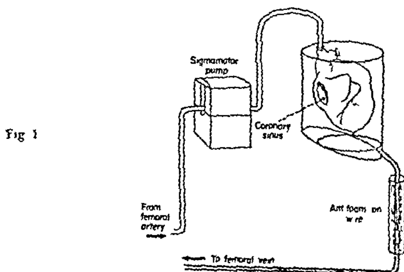
METHOD

The brachiocephalic artery of a donor dog was catheterized and arterial blood delivered to it from the femoral artery of a perfusing dog A constant controlled flow was provided by placing a Sigma motor pump on the perfusing line Venous blood was returned to the donor animal via a defoaming chamber where the blood level was adjusted to prevent pulmonary air embolization to the donor dog When these arrangements were complete the cavae were occluded and the aorta clamped distal to the brachiocephalic artery Perfusion was begun and the beating heart was excised and suspended in a large plastic chamber (Fig 1)

Simultaneously paired arterial and coronary sinus samples were obtained at various time intervals These were analyzed for pH, hematocrit, hemoglobin, potassium oxygen, carbon dioxide, glucose, lactic acid, and pyruvic acid After an adequate number of samples had been taken to provide satisfactory baseline data, potassium citrate arrest was induced and following a constant time interval of arrest the sampling and analysis were repeated

At the end of each experiment the exact flow was measured and the heart weighed after removal of fat and vessels

Fig 1



RESULTS

Twenty-one isolated hearts were studied in order to provide an adequate sample of their baseline metabolism The mean values of the utilization of the various metabolites are given below These values are given in terms of milliliter or milligrams per 100 gm of heart muscle per minute Their molar values are also included since all inter reactions occur in molar equivalents

METABOLITE	TOTAL NO OF SAMPLES	UTILIZATION Mg or ml /100 gm /min	Molsx10 ⁵/100 gm /min
Oxygen	47	3 8	16 95
Carbon Dioxide	46	3.3	14 73
Glucose	45	6 12	3.38
Lactic Acid	34	11	12
Pyruvic Acid	35	053	.56

Six mols of oxygen are required to completely oxidize 1 mol of glucose Glycolysis of a hexose, e g glucose of glycogen, at one point produces two trioses, one of which is pyruvic acid From our data, where the mean molar value of pyruvic acid excretion is substracted from that of glucose a value of *2 83 mx10 ⁵/100 gm /min* is obtained This value is very nearly ⅙ of the mean molar oxygen utilization value This suggests that glucose serves almost solely as the substrate for oxidative carbohydrate metabolism Figure 2 is a chart showing the linear regressions of the various metabolites as a function of time Except for lactic acid these straight lines have little slope Statistical analysis confirms this observation That is, the slopes of the lines do not differ significantly from 0 the slope of a horizontal line This also applies to the line of lactic acid utilization despite its apparent steepness Thus the utilization of any of the metabolites analyzed is probably proceeding at a relatively steady rate regardless of time

The mean utilization values of lactic acid and pyruvic acid are very small and the limits of the 95% confidence interval lie above and below a zero value for their utilization Statistically these values are not significantly different from 0 utilization It is reasonable to assume therefore, that there

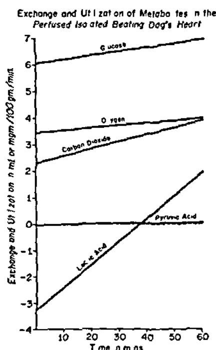


Fig 2

is no significantly consistent utilization of these two metabolites

Most authors state that the extraction of glucose and lactic acid in a heart working at a steady rate is directly proportional to the arterial con centrations of these metabolites Correlation coefficients of the utilization of glucose and lactic acid on their arterial concentration in our studies are very small and give t values which are not significant

Calculation of the energy produced from the glucose metabolized gives a value of 2 3 $\times$ 10^{-2} cal per 100 gm of heart per minute This is a low value when compared with those calculated on the basis of glucose and lactic acid utilization published by others in preparations where the heart is pumping a load against a peripheral resistance

Our observations are not yet complete in studies of the heart in induced arrest In this situation the heart is carrying out only such processes as will maintain cellular functional integrity This appears to result in a massive decrease in the values of energy production based on the quantity of metabolites utilized

DISCUSSION

Three things are apparent from our baseline studies in the preparation described First there was no consistent significant utilization of lactic acid as a metabolic substrate Most authors report lactic acid utilization in the many different preparations studied This substrate is said[6] to con tribute from 30% to 100%[2] of the total carbohydrate oxidative metabolism We have been unable to confirm this in our preparation Second glucose and lactic acid utilization in a stable working state was not related to arterial glucose concentration A commonly stated feature of cardiac metabolism during a steady working state is the utilization of glucose and lactic acid in direct proportion to the arterial concentration of these compounds This is difficult to understand for the heart would then become the end organ for the blood level of a compound In our preparation we found no sig nificant correlation

Glucose

$t = 1\ 4196 \quad n = 43 \quad 4 < 2P < 2)$ Lactate $t = 5863 \quad n = 32 \quad 1\ 0 < 2P < 1\ 0)$

Third in these hearts which were beating but not pumping a load against a resistance the energy production work load was low Preliminary studies in the induced arrested heart reveal an even lower level of energy pro duction derived primarily from anaerobic carbohydrate metabolism

CONCLUSION

1 There is no consistent significant utilization of lactic acid or pyruvic acid in the isolated perfused beating dog heart

2 In this preparation there is no significant correlation between glucose and lactic acid utilization and arterial concentration in the steady non working beating heart

5 Energy production is considerably reduced as compared with the value of hearts *in vivo* An even greater reduction in energy production occurs in the arrested heart

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THE INFLUENCE OF CARDIAC OUTPUT, AORTIC PRESSURE AND HEART RATE ON MYOCARDIAL OXYGEN UTILIZATION*

G H WELCH, JR, S J SARNOFF, E BRAUNWALD, W N STAINSBY, R B CASE, AND R MACRUZ

The purpose of this study was threefold 1) to demonstrate that myocardial oxygen utilization is dependent upon the type of work that the heart is called upon to perform, not upon the quantity of work to be done 2) To show that a consistent relationship exists between myocardial oxygen consumption and a factor present in all types of myocardial work, however induced, this being the area under the systolic pressure curve, referred to as the tension time index, or T T I [1] 3) To point out the importance of Laplace s law in the interpretation of these phenomena

METHOD

Mongrel dogs of both sexes weighing from 19 6 to 34 0 kg were premedicated with 2 mg /kg of morphine sulfate intramuscularly and anesthetized with approximately 48 mg /kg of chloralose and 480 mg /kg of urethane intravenously The preparation (Fig 1) consisted of an isolated supported, canine heart,[2] the left ventricle of which ejected its blood through a Starling resistance (SR), turbine flowmeter (PET),[3] and arterial densitometer (DA)[4] into a reservoir (RES) from which blood was returned

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to the left atrium through a second Starling resistance (IR) The brachyocephalic and left subclavian arteries and the pulmonary veins were ligated, making this a closed circuit except for coronary artery flow

The right heart received no blood other than that from the coronary veins, ligature of the cavae and azygus having been done This completely mixed coronary venous blood was ejected via the pulmonary artery through a venous densitometer (DV)[4] and flowmeter (ROT)[5] to the jugular veins of a second or support dog (SD) Biochemically normal blood from the femoral arteries of this support dog was simultaneously returned to the reservoir system, the level of blood in the reservoir being maintained at a constant height by means of a float which electrically controlled the opening of the solenoid valve (SOL) The reservoir was filled with donor blood acquired from heparinized, lightly pentothalized donors

Aortic pressures, cardiac output, and heart rate were independently controlled by appropriate adjustments of the outflow resistance, the inflow resistance, and an electrical pacemaker on the right atrium Blood temperature was held constant Continuous recordings were made of heart rate, coronary and aortic flows, arterial and coronary venous oxygen saturations, and pressures in the left and right atria, pulmonary artery, and aorta Intermittently obtained data included 100 mm/sec tracings of aortic, full left ventricular, and left ventricular end diastolic pressures, Van Slyke analysis for O_2 and CO_2 contents on arterial and coronary venous samples,[6] and hematocrits

Myocardial O_2 consumption in cc/min (VO_2) was calculated as the product of coronary flow in cc/min and the coronary arteriovenous oxygen difference Left ventricular minute work in kilogram meters was calculated as the product of cardiac output (aortic flow plus coronary flow) in liters per minute divided by 100 and the planemetrically integrated mean systolic pressure in cm H_2O The area under the systolic portion of the aortic pressure curve, referred to hereafter as the tension time index (T T I) per beat in mm Hg seconds, is the product of mean systolic pressure and duration of systole External efficiency is the ratio of minute work in kg M to the product of VO_2 and the conversion factor of 2 06[7]

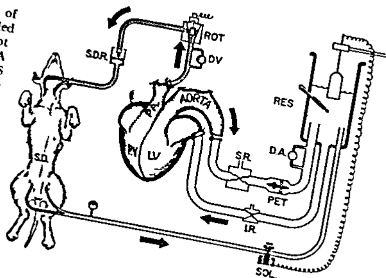


Fig 1 Schematic drawing of the preparation SR air filled Starling resistance PET Potter electroturbinometer DA arterial densitometer RES reservoir IR water filled inflow Starling resistance DV venous densitometer ROT rotameter SDR support dog reservoir SD support dog SOL solenoid valve electrically operated by microswitch at top of reservoir float Courtesy of Am J Physiol[8]

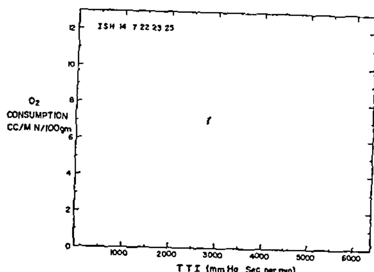


Fig 2 Graph illustrating the close correlation bet een tension time index (T T I) and myocardial oxygen consumption in cc/min/100 gm of left ventricular muscle in 5 isolated supported heart experiments under variable experimental circumstances

RESULTS

Twenty of 27 attempted experiments yielded useful information The results are divided into 3 groups

Group 1 The influence of cardiac output on myocardial O_2 utilization Increasing left ventricular work as much as 700% by stepwise augmentation of cardiac output while mean aortic pressure and heart rate were held constant at any given magnitude caused only a 53% increase in VO_2 with a resultant striking increase in external myocardial efficiency The T T I correlated well with the VO_2 (Fig 2)

Group 2 The influence of aortic pressure on myocardial O_2 utilization Increasing left ventricular work by several hundred per cent by stepwise elevations of aortic pressure while cardiac output and heart rate were held constant caused a parallel increase in VO external efficiency remaining unchanged T T I again correlated well with VO_2 (Fig 2)

Group 3 The influence of heart rate on myocardial O_2 utilization With mean aortic pressure held constant at 120 mm Hg and the heart rate at 120/min cardiac output was progressively augmented from 1 2 to 4 9 L/min This was then repeated at heart rates of 160/min and again at 200/min A higher heart rate at any given work level was accompanied by an increased myocardial VO_2 with a diminished external efficiency The T T I again correlated well with VO (Fig 2)

DISCUSSION

That the oxygen requirements of flow work are not as great as those of an equal quantity of pressure work has been observed previously in the isolated heart heart lung preparation and the dog with a complete circulation[8 9 10 1 12 13] It has also been shown that acceleration of heart rate increases the VO[4] The above data are consonant with these observations It would seem however that since VO varies concomitantly with T T I no matter which of the heart s hemodynamic parameters is varied — and since VO can vary widely at any given filling pressure[13] that VO is determined by events occurring subsequent to the onset of contraction (as reflected by T T I) rather than by the filling pressure prior to contraction Furthermore if the T T I is taken as an *index* of myocardial

wall tension (in that no method of actual measurement exists)[1] then Laplace s law as applied to the heart[14] is seen to take on an important role in the relationship between VO and myocardial failure with cardiac dilatation Laplace s law states that for a cylinder P equals T/R where P equals intraluminal pressure T equals wall tension and R equals radius Thus to develop any given intraluminal pressure at the same stroke volume and heart rate a heart with a large radius must develop a greater wall tension or T T I than a heart with a small radius and hence the oxygen consumption of the dilated heart will be greater

The importance of these phenomena becomes apparent in connection with myocardial hypoxia occurring in hypertension coronary artery disease aortic stenosis and tachycardia or in the patient with cardiac dilatation either because of disease or following intracardiac surgery

SUMMARY AND CONCLUSIONS

1 With an isolated metabolically supported heart preparation it was possible to independently control and vary cardiac output aortic pressure and heart rate

2 There was no straight forward relationship between myocardial work *per se* and the heart s consumption of oxygen

3 Increasing work markedly by augmenting cardiac output caused only a slight rise in myocardial oxygen consumption while increasing work by elevating aortic pressure caused a parallel increase in oxygen consumption When heart rate was accelerated at a constant work level oxygen con sumption increased despite the lack of increase in work load

4 The heart s oxygen consumption bore a close relationship to the area under the aortic systolic pressure curve as expressed by T T I

5 The application of Laplace s law to the relationship between the heart s oxygen consumption and myocardial failure was briefly discussed

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MYOCARDIAL METABOLISM DURING HYPOTHERMIA WITH CAVAL OCCLUSION AND LOW FLOW CORONARY PERFUSION*

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The perfusion of warmed, oxygenated, heparinized blood through the hypothermic heart during periods of inflow tract occlusion has decreased the incidence of ventricular fibrillation and extended the use of hypothermia for open heart surgery [1 2 3 4]

To evaluate the effectiveness of low flow coronary perfusion, we have performed metabolic studies of the hypothermic non fibrillating and fibrillating canine myocardium during periods of inflow tract occlusion with and without coronary perfusion

METHOD

Thirty five mongrel dogs were anesthetized with sodium pentothal and surface cooled to rectal temperatures of 26 to 30 1°C The dogs were ventilated with 100% O_2 during cooling and hyperventilated prior to inflow tract occlusion Through a right fifth interspace thoracotomy, the superior and inferior vena cavae, aorta, and both lung roots were mobilized Following inflow tract occlusion respirations were stopped, a right atriotomy was performed and the coronary sinus cannulated with a Foley catheter After varying periods of inflow tract occlusion, both lung roots and the aorta were occluded and a curved needle placed into the aorta between the clamp and aortic valve for perfusion of the coronary arteries Blood was withdrawn from donor dogs the morning of the experiment and heparinized The donor blood was then transfused into the experimental dog during the thoracotomy, while arterial blood was being removed from it

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Supported in part by United States Public Health Grant G A 470 and the Clinical Traineeship of the National Heart Institute
With the technical assistance of Dr Augusta McCoord, Carol Higley and Marjorie Metzler

and heparinized for use as a perfusate Preliminary studies showed that such perfusate blood was 95 to 100% saturated with oxygen The blood was kept in a water jacket at a temperature of 36 to 38°C until used Plasma was prepared from heparinized blood the day before the experiment and stored at a temperature of 4°C until it was warmed to 36 to 38°C for use as a perfusate Assisted gravity perfusion was begun at flow rates of 1 5 to 3 2 cc /kg body weight/min for 6 to 12 minute periods Samples of blood were taken from the perfusate, the aorta and, in some instances, from the coronary sinus just before inflow tract occlusion During perfusion continuous samples of blood were obtained from the coronary sinus The length of the collection periods was varied to insure obtaining enough blood for analysis The samples were analyzed for hydrogen ion, CO_2, lactic acid, glucose and potassium concentrations by accepted methods

Data were obtained from the following groups of dogs

Group 1 — 10 dogs No coronary perfusion Coronary sinus samples were obtained during a 12 minute period of inflow tract occlusion alone, since insufficient blood for analysis was obtained if the aorta and lung roots were also occluded [5]

Group 2 — 5 dogs Coronary perfusion with plasma after a 6 to 7 minute period of inflow tract occlusion

Group 3 — 5 dogs Coronary perfusion with blood These dogs were Group 1 dogs which had aortic and root of lung occlusion with coronary perfusion initiated after a 12 minute period of inflow tract occlusion

Group 4 — 6 dogs Coronary perfusion with blood after a 4 to 6 5 minute period of inflow tract occlusion

Group 5 — 5 dogs Coronary perfusion with blood starting within 1 minute of inflow tract occlusion

Group 6 — 4 dogs Coronary perfusion with blood Fibrillation had started before inflow tract occlusion

Group 7 — 3 dogs Coronary perfusion with blood Fibrillation started just before onset of perfusion and after inflow tract occlusion

Group 8 — 2 dogs Coronary perfusion with plasma Fibrillation started just before onset of perfusion

RESULTS

pH changes The pH of coronary sinus blood decreased during inflow tract occlusion alone Perfusion with plasma resulted in continued decreases in pH Perfusion with blood after 4 to 12 minutes of inflow tract occlusion resulted in the return of the pH toward normal levels If the perfusion of blood was started within 1 minute of inflow tract occlusion, the pH remained stable at levels near the control coronary sinus value The decrease in pH of coronary sinus blood was most marked during ventricular fibrillation Plasma perfusion failed to modify this, but whole blood resulted in a moderate increase

Bicarbonate changes. Coronary sinus bicarbonate levels decreased during inflow tract occlusion In general, further decreases were noted during plasma perfusion, to levels lower than that in the perfusate In general, the levels rose during periods of blood perfusion, started after 4 to 12 minutes of inflow tract occlusion, and remained at levels above that of the perfusate If blood perfusion was started within one mintue of inflow tract

occlusion the bicarbonate levels remained higher than those of the perfusate The perfusion of plasma during fibrillation resulted in levels lower than those of the perfusate, but during blood perfusion they remained at levels higher than that of the perfusate

Lactic acid changes. Coronary sinus lactic acid levels rose during inflow tract occlusion alone, and continued to rise during plasma perfusion Lactic acid values decreased to near the control level with perfusion of whole blood, which was begun after 4 to 12 minutes of inflow tract occlusion If the perfusion was started within one minute of inflow tract occlusion the lactic acid concentration remained at low levels and in two instances fell below that of the perfusate The lactic acid concentrations were high during fibrillation, remained high with plasma perfusion and decreased toward control levels with blood perfusion

Potassium changes. The coronary sinus potassium concentration remained remarkably constant during inflow tract occlusion, with or without the perfusion of blood or plasma, if the ventricles did not fibrillate With fibrillation the potassium levels consistently rose The perfusion of plasma resulted in continued high levels, but the perfusion of blood resulted in decreases to below that of the perfusate

Glucose changes In general, the coronary sinus glucose concentration was lower than that in the control aortic sample During perfusion with plasma or blood and with or without fibrillation, the glucose levels remained below those of the perfusate

DISCUSSION

Inflow tract occlusion of the hypothermic heart produces a myocardial acidosis, as indicated by the decrease in the pH of the coronary sinus blood in these experiments The rather marked decrease in pH, in the presence of minor decreases in the bicarbonate concentration in the coronary sinus blood, suggests that the acidosis is due to both the accumulation of CO_2 and the production of lactic acid (The myocardium normally utilizes lactic acid Coronary sinus levels in excess of arterial levels have been recorded only under conditions of myocardial hypoxia)[6, 7] Furthermore, if plasma is perfused through the myocardium, an even more marked acidosis resulted Therefore, it would seem that the acidosis is secondary to the deficiency of perfused oxygenated erythrocytes with their oxygenating and buffering abilities

In these experiments the perfusion of fresh, warmed, heparinized oxygenated blood through the myocardium following periods of inflow tract occlusion could return the coronary sinus pH, lactic acid concentrations and CO_2 content toward or actually to normal levels (Fig 1) Furthermore if the perfusion were started within one minute of inflow tract occlusion the coronary sinus pH, lactic acid concentration and CO_2 content could be maintained at stable normal levels, including in some instances a positive lactic acid balance across the myocardium (Fig 1) This protection of the myocardium was accomplished with below normal perfusion rates The normal canine coronary blood flow at temperatures of 26 to 27°C is approximately 4 cc/kg body weight/min (calculated from Edwards data)[8] Our average perfusion rate was 2 6 cc./kg body weight/min

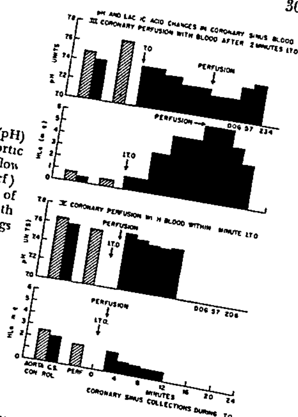


Fig 1 The hydrogen ion concentration (pH) and lactic acid (HLa) concentrations in aortic and coronary sinus (C.S.) blood prior to inflow tract occlusion (controls) in the perfusate (perf) and in the coronary sinus during periods of inflow tract occlusion (I T O) without and with coronary perfusion Examples of results in dogs from Group 3 and 5

Fibrillation and inflow tract occlusion caused more marked changes in the coronary sinus pH, CO_2 and lactic acid levels, than inflow tract occlusion alone Fresh, warmed, oxygenated, heparinized blood returned the myocardial metabolism to or toward more normal levels

Although the myocardium did not consistently lose or gain potassium during inflow tract occlusion, there was a marked loss during fibrillation, as has been previously observed[9,10] Although potassium did not apparently reenter the myocardial cell with the perfusion of plasma during fibrillation, it did enter the myocardium with the perfusion of blood during fibrillation This is further evidence of the ability of blood perfusion to improve myocardial metabolism during inflow tract occlusion

SUMMARY

1 The coronary perfusion of warmed oxygenated heparinized blood at below normal coronary flow rates can correct or prevent the acidosis occurring during inflow tract occlusion

2 The same type of perfusion tends to correct the more marked acidosis occurring with ventricular fibrillation and can reverse the negative myocardial potassium balance occurring with ventricular fibrillation

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*RESTORATION OF FUNCTION OF THE REFRIGERATED HEART**

WATTS R WEBB AND HECTOR S HOWARD

The principle of reducing the temperature of a tissue during a period of acute ischemia has been well established and has received wide use both experimentally and clinically The present study was designed to determine whether the isolated mammalian heart can survive for prolonged periods at low temperatures and return to adequate function

METHOD

Hearts from adult mongrel dogs were isolated with inflow and outflow occlusion and perfused with lactated Ringer's solution until all blood and plasma had been flushed from the heart [1] The heart was excised and immersed in chilled Tyrode's solution with 10% serum added according to the formula of Gross, Bill and Peirce[2] and maintained at 4°C for periods ranging from 6 to 72 hours Subsequently, they were transplanted to the necks of large recipient dogs, anastomosing the distal carotid artery of the host to the donor brachiocephalic artery and the proximal end of the recipient jugular vein to the left pulmonary artery of the donor In addition, the proximal carotid artery of the host was anastomosed to the left auricular appendage [3] The large inflow from this carotid artery into an area of low resistance served as a further index of cardiac function Before restoration of flow, the recipient dog was heparinized

Defibrillation, when needed, was accomplished by an electric shock of 180 volts and 0 1 second duration After restoration of cardiac action, the hearts which functioned satisfactorily were implanted in a subcutaneous pocket for observation of survival

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Supported by U S Public Health Service Grant No H 2806

A second series was treated similarly except that the heart was not perfused to flush all blood elements from the coronary capillary bed. In a third series, to test whether a heart that had been acutely stopped might remain in better nutritional status during hypothermia, the donor dogs were heparinized with 1 mg. heparin/kg. body weight and the hearts stopped with potassium citrate without being irrigated. In the fourth series the dogs were heparinized with 10 mg. heparin/kg. before potassium citrate cardioplegia.

OBSERVATIONS

On initial perfusion of the donor hearts with lactated Ringer's solution, 18 of 21 hearts stopped after a period of approximately 5 to 10 minutes without fibrillation while 3 fibrillated. The development of fibrillation did not appear to be a significant factor in the results.

As J. T. Webb[4] and co-workers have shown that within 5 minutes at normal temperatures there is a 36% loss in oxygen uptake of tissue slices of cardiac muscle, it appears important to cool the hearts as quickly as possible. Usually the hearts could be removed, separated from the lung, and placed in the chilled Tyrode's solution in less than 5 minutes after completion of the perfusion maneuver.

Several factors appeared to be of some importance in obtaining good results. Dilatation of the heart at any stage of the procedure is deleterious. Rewarming occurred in part during the period of 15 to 20 minutes required for the anastomoses to be accomplished. Usually a further period of approximately 5 minutes of blood flow and very gentle massage sufficed for a return to normal temperature.

Adrenalin and calcium were most valuable in improving myocardial tone. These hearts were extremely sensitive to drugs, particularly calcium, as often only 0.5 cc. of 10% calcium chloride was sufficient to restore tone and more than this would cause systolic tetany which was much more difficult to control than flaccidity or fibrillation. Most hearts on restoration of tone fibrillated though some restarted with a coordinated rhythm. Defibrillation could be accomplished easily by electric shock in the presence of firm cardiac tone and slow coarse fibrillation. The conduction system appears to be the most susceptible portion of the heart. Usually at first there was complete A-V dissociation and it might require an hour for a normal sinus rhythm to return. During this period the ventricular rate would be 60 to 200 per minute with auricular rates of 120 to 220.

RESULTS

In the perfused series, 9 hearts which were transplanted after a period of 6 to 8 hours all showed excellent function. One heart which did not do well had a constricted arterial anastomosis with probably inadequate coronary blood flow. Each of the others was able to maintain the work load of its own coronary circulation and also the load imposed by the inflow of the proximal carotid artery into the left atrium. Measurements of the carotid artery flow into a low pressure system averaged about 40 cc./kg./min. As the recipient dog was usually 3 to 5 times as large as the donor, flow into the transplanted heart would be 120 to 200 cc./kg. donor dog weight and thus approximate full cardiac output.

Table 1. Refrigeration at 4°C Perfused Heart Series

HOURS	NUMBER	TONE	FUNCTION
6-8	10	Excellent	Excellent
18-24	6	Good	Fair
28-36	3	Fair (2)	Fibrillation (2)
54	1	Slight	0
72	2	0	0

Of the 6 perfused hearts which were refrigerated from 18 to 24 hours, it was possible to restore a beat in 5, but only one beat strongly. This one at 18½ hours was able to maintain the added load of the proximal carotid inflow for a period of approximately 30 minutes before starting to dilate and fail. Two hearts at 28 hours were able to develop fairly good tone and fibrillate but could not be restored to a normal beat. One heart at 36 hours remained completely flaccid. One at 53 hours did regain tone but did not fibrillate or function otherwise. Two hearts at 72 hours showed no signs of viability.

The 3 nonperfused hearts at 6 and 8 hours developed good tone and fibrillated but could not be resuscitated to a strong beat. At 16 hours one showed some contraction but was unable to pump its coronary inflow. The hearts at 17 and 19 hours showed only fair return of tone, one could not be defibrillated and the other beat briefly with poor, incoordinated contractions. After 24 hours, no tone or fibrillation was achieved.

In the series using hearts heparinized with 1 mg heparin and cardioplegia with potassium citrate, 3 were able to regain good tone but only a weak beat after 8 hours. After 15 hours of refrigeration, tone was poor and no coordinated beat developed. At 23 hours the heart remained cyanotic, developed very poor tone and showed only a few fibrillations. In each of these multiple clots were noted in the coronary vessels, and the myocardium showed many persistent diffuse ischemic areas.

The heavily heparinized hearts with potassium citrate cardioplegia, however, did excellently at 6 to 8 hours though poorer at later intervals as

Table 2. Refrigeration Non Perfused — Cardioplegia

	HOURS	NO.	TONE	FUNCTION
1 mg Heparin	[illegible]	3	[illegible]	Poor
	[illegible]	1	[illegible]	Fibrillation
	[illegible]	1	[illegible]	Fibrillation
10 mg Heparin	[illegible]	4	[illegible]	[illegible]llent

expected. As well as could be judged, these did as well as, though no better than, the perfused series, which presumably had developed an oxygen debt prior to being refrigerated.

The experimental hearts continued to beat in the subcutaneous pockets for periods up to 2 days. Studies by Marcus, Wong and Luisada[5] on older dogs likewise found a survival period for the adult dog heart of approximately 2 days though puppy hearts may live much longer.

DISCUSSION

While there have been no previous studies on refrigeration of the heart itself, there has been a great deal of study on other tissues such as red blood cells, spermatozoa, bacteria, etc. which can be maintained in the deep freeze and restored at will. Bernheim and Bernheim[6] found that heart tissue slices can be kept at 5°C. for 30 minutes without interfering with subsequent metabolic studies but apparently did not test longer refrigeration periods.

As far as the human heart is concerned, J. B. E. Baker[7] used the Langendorff perfusion system on the hearts of fetuses of 16 to 24 weeks gestation, starting ¾ to 2¼ hours after cesarean section. Using oxygenated Tyrode's solution at 29°C. he was able to restore cardiac contractions in all 9, though there was no measurement of functional ability. Ossinowsky[8] and Kuliabko[9] have claimed to revive children's hearts up to 28 and 30 hours after death, though again no measurement is given of functional capacity except that any contraction of any portion of the heart was recorded as a success. Kountz,[10] in studies upon adult human hearts obtained at autopsy, found that he could nearly always restore cardiac contractions within 30 minutes, frequently within 2 hours after death, and in a few cases up to 6 hours after death. Nothing was done to these hearts in the way of heparinization or perfusion to remove all the blood elements from the coronary capillary bed prior to death or immediately thereafter.

Demonstration that the heart can be maintained viable and functional for periods of at least 8 hours by refrigeration in a nutrient medium has been of great value to us in experimental work in cardiac and cardiopulmonary transplants. When the problems of immunology are solved and transplantation becomes a clinical possibility, it will presumably require several hours for obtaining the heart, preparation of the recipient and total reimplantation. The practices outlined above would seem to make cardiac transplantation completely feasible so far as this time element is concerned.

SUMMARY

1. Normal adult canine hearts, after having all blood flushed from the coronary system, can be preserved for periods of 6 to 8 hours in a nutrient solution at 1°C. and restored to an efficient functional state.

2. Control hearts which had not been perfused did not return to adequate function after refrigeration.

3. Hearts stopped with potassium citrate with blood remaining within the chambers and coronary vessels do poorly following refrigeration, as intravascular clotting occurred in spite of usual degrees of heparinization.

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Of the 6 perfused hearts which were refrigerated from 18 to 24 hours *it was possible to restore a beat in 5, but only one beat strongly* This one at 18½ hours was able to maintain the added load of the proximal carotid inflow for a period of approximately 30 minutes before starting to dilate and fail Two hearts at 28 hours were able to develop fairly good tone and fibrillate but could not be restored to a normal beat One heart at 36 hours remained completely flaccid One at 53 hours did regain tone but did not fibrillate or function otherwise Two hearts at 72 hours showed no signs of viability

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	23	1	Poor	Fibrillation
10 mg	6 8	4	Excellent	Excellent
Heparin	17	1	Excellent	Good
	21	1	Good	Poor

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As far as the human heart is concerned, J. B. E. Baker[7] used the Langendorff perfusion system on the hearts of fetuses of 16 to 24 weeks gestation, starting 3/4 to 2 1/4 hours after cesarean section. Using oxygenated Tyrode's solution at 29°C. he was able to restore cardiac contractions in all 9, though there was no measurement of functional ability. Ossinowsky[8] and Kuliabko[9] have claimed to revive children's hearts up to 28 and 30 hours after death, though again no measurement is given of functional capacity except that any contraction of any portion of the heart was recorded as a success. Kountz,[10] in studies upon adult human hearts obtained at autopsy, found that he could nearly always restore cardiac contractions within 30 minutes, frequently within 2 hours after death, and in a few cases up to 6 hours after death. Nothing was done to these hearts in the way of heparinization or perfusion to remove all the blood elements from the coronary capillary bed prior to death or immediately thereafter.

Demonstration that the heart can be maintained viable and functional for periods of at least 8 hours by refrigeration in a nutrient medium has been of great value to us in experimental work in cardiac and cardiopulmonary transplants. When the problems of immunology are solved and transplantation becomes a clinical possibility, it will presumably require several hours for obtaining the heart, preparation of the recipient and total reimplantation. The practices outlined above would seem to make cardiac transplantation completely feasible so far as this time element is concerned.

SUMMARY

1. Normal adult canine hearts, after having all blood flushed from the coronary system, can be preserved for periods of 6 to 8 hours in a nutrient solution at 4°C. and restored to an efficient functional state.

2. Control hearts which had not been perfused did not return to adequate function after refrigeration.

3. Hearts stopped with potassium citrate with blood remaining within the chambers and coronary vessels do poorly following refrigeration, as intravascular clotting occurred in spite of usual degrees of heparinization.

Table 1 Refrigeration at 4°C Perfused Heart Series

HOURS	NUMBER	TONE	FUNCTION
6 8	10	Excellent	Excellent
18 24	6	Good	Fair
28 36	3	Fair (2)	Fibrillation (2)
54	1	Slight	0
72	2	0	0

Of the 6 perfused hearts which were refrigerated from 18 to 24 hours, it was possible to restore a beat in 5, but only one beat strongly This one at 18½ hours was able to maintain the added load of the proximal carotid inflow for a period of approximately 30 minutes before starting to dilate and fail Two hearts at 28 hours were able to develop fairly good tone and fibrillate but could not be restored to a normal beat One heart at 36 hours remained completely flaccid One at 53 hours did regain tone but did not fibrillate or function otherwise Two hearts at 72 hours showed no signs of viability

The 3 nonperfused hearts at 6 and 8 hours developed good tone and fibrillated, but could not be resuscitated to a strong beat At 16 hours one showed some contraction but was unable to pump its coronary inflow The hearts at 17 and 19 hours showed only fair return of tone, one could not be defibrillated and the other beat briefly with poor, incoordinated contractions After 24 hours, no tone or fibrillation was achieved

In the series using hearts heparinized with 1 mg heparin and cardioplegia with potassium citrate, 3 were able to regain good tone but only a weak beat after 8 hours After 15 hours of refrigeration, tone was poor and no coordinated beat developed At 23 hours the heart remained cyanotic, developed very poor tone and showed only a few fibrillations In each of these, multiple clots were noted in the coronary vessels, and the myocardium showed many persistent diffuse ischemic areas

The heavily heparinized hearts with potassium citrate cardioplegia, however, did excellently at 6 to 8 hours, though poorer at later intervals as

Table 2 Refrigeration Non Perfused — Cardioplegia

	HOURS	NO	TONE	FUNCTION
1 mg	6 8	3	Good	Poor
Heparin	16	1	Good	Fibrillation
	23	1	Poor	Fibrillation
10 mg	6 8	4	Excellent	Excellent
Heparin	17	1	Excellent	Good
	24	1	Good	Poor

expected As well as could be judged these did as well as though no better than the perfused series which presumably had developed an oxygen debt prior to being refrigerated

The experimental hearts continued to beat in the subcutaneous pockets for periods up to 2 days Studies by Marcus Wong and Luisada[5] on older dogs likewise found a survival period for the adult dog heart of approximately 2 days though puppy hearts may live much longer

DISCUSSION

While there have been no previous studies on refrigeration of the heart itself there has been a great deal of study on other tissues such as red blood cells spermatozoa bacteria etc which can be maintained in the deep freeze and restored at will Bernheim and Bernheim[6] found that heart tissue slices can be kept at 5°C for 30 minutes without interfering with subsequent metabolic studies but apparently did not test longer refrigeration periods

As far as the human heart is concerned J B E Baker[7] used the Langendorff perfusion system on the hearts of fetuses of 16 to 24 weeks gestation starting ¾ to 2¼ hours after cesarean section Using oxygenated Tyrode s solution at 29°C he was able to restore cardiac contractions in all 9 though there was no measurement of functional ability Ossinowsky[8] and Kuliabko[9] have claimed to revive children s hearts up to 28 and 30 hours after death though again no measurement is given of functional capacity except that any contraction of any portion of the heart was recorded as a success Kountz[10] in studies upon adult human hearts obtained at autopsy found that he could nearly always restore cardiac contractions within 30 minutes frequently within 2 hours after death and in a few cases up to 6 hours after death Nothing was done to these hearts in the way of heparinization or perfusion to remove all the blood elements from the coronary capillary bed prior to death or immediately thereafter

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SUMMARY

1 Normal adult canine hearts after having all blood flushed from the coronary system can be preserved for periods of 6 to 8 hours in a nutrient solution at 4°C and restored to an efficient functional state

2 Control hearts which had not been perfused did not return to adequate function after refrigeration

3 Hearts stopped with potassium citrate with blood remaining within the chambers and coronary vessels do poorly following refrigeration as intravascular clotting occurred in spite of usual degrees of heparinization

4 If massive doses of heparin (10 mg/kg) were used prior to cardioplegia and refrigeration, the results seem to be as good as with removal of all blood elements by perfusion

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CARDIAC ARRHYTHMIAS AND VENTRICULAR FIBRILLATION RESULTING FROM RAPID REVERSAL OF HYPERCARBIA*

Archer S Gordon Philip W Andrews, Frank E Adrian and E J Beattie Jr

Severe cardiac arrhythmias and ventricular fibrillation occurring during or immediately after surgery pose a grave threat Numerous etiologic factors have been incriminated in these conditions but the exact mechanism frequently remains undetermined One of the factors known to play a role in some cases is carbon dioxide retention

Recently Brown and Miller and coworkers [1, 2, 3] have produced serious cardiac arrhythmias and ventricular fibrillation in dogs by the rapid reversal of hypercarbia following prolonged breathing of 30 to 40% exogenous carbon dioxide These cardiac effects were associated with a marked hyperkalemia Similar observations have been reported by Sealy Young and Harris [4, 5]

*From the Dept of Surgery Presbyterian St Lukes Hosp Chicago and the Dept of Clinical Science University of Illinois College of Medicine Supported by a research grant from the Army Chemical Warfare Laboratories Edgewood Maryland

In order to relate these observations to clinical surgery, it is necessary to determine whether comparable amounts of endogenous carbon dioxide can accumulate and produce the same effects Accordingly, this study was undertaken to assess the role of rapid reversal of endogenous hypercarbia in the production of cardiac arrhythmias and ventricular fibrillation

METHOD

Ten healthy dogs, anesthetized with nembutal, rebreathed continuously into a large rubber bag for 90 minutes Anoxia was prevented by replacing the oxygen as utilized At the end of this time the bag was removed and the animals were hyperventilated with room air for one minute after which they breathed air spontaneously Lead II ECG's and blood pressure were recorded continuously on a Sanborn Twin Viso Recorder Blood samples were drawn periodically from the carotid artery, as often as every minute during the periods of rapid change CO_2 content, pH and plasma potassium were *determined on these specimens, and* pCO₂ *values were calculated* from the nomogram of Singer and Hastings

In a second series of 10 dogs, the same procedure was repeated, with the addition of 0 05 mg /kg of epinephrine given intravenously at the beginning of the rapid reversal of hypercarbia

RESULTS

Figure 1 summarizes the mean results on the first series During the period of CO_2 retention, arterial pCO_2 rose gradually from 38 to 247 mm Hg at 90 minutes The pH fell from 7 42 to 6 82 during the same time There was an initial transient rise in plasma potassium within 10 minutes after the onset of rebreathing The potassium then gradually returned to the control level where it remained until the time of CO_2 elimination

At 90 minutes, hyperventilation with room air resulted in dramatic changes The pCO_2 dropped precipitously to a subnormal level (25 mm Hg) and the pH made a sudden alkalotic swing to 7 50 within 2 to 3 minutes There was a simultaneous abrupt rise in plasma potassium from 4 6 to 5 7 mEq/L at 4 minutes The potassium returned to normal levels within 5 minutes whereas the pH and pCO_2 required longer

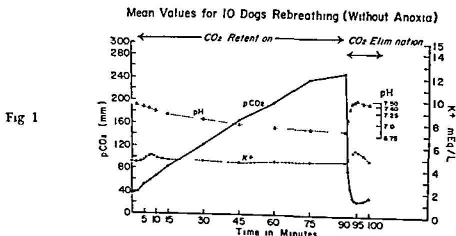


Fig 1

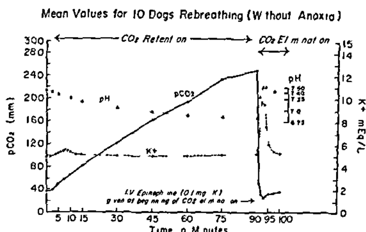


Fig 2

During the initial period of carbon dioxide retention, all dogs showed a sinus tachycardia, tachypnea and rising blood pressure After one hour the marked elevation in pCO_2 resulted in a reversal of this trend with a progressive slowing of pulse and respiration and gradual decrease in blood pressure *At the time of rapid CO_2 elimination* cardiac arrhythmias occurred in every dog The ECG s showed bizarre patterns including nodal arrhythmias, A V dissociation, ventricular extrasystoles and ventricular tachycardia There was a brief pulse acceleration and systolic pressure rise followed in most cases by a relative bradycardia and hypotension Nine dogs survived the experiment and eventually returned to a normal sinus rhythm

In one dog, the ventricular tachycardia progressed into ventricular fibrillation 4 minutes after the reversal of hypercarbia The serum potassium at this time was 6 8 mEq/L The highest pCO_2 recorded in this series was 340 mm Hg and the lowest pH was 6 8

Essentially the same results were noted in the second series of *10* dogs, which received 0 05 mg /kg of epinephrine intravenously at the time of reversal of hypercarbia (Fig 2) However, there was one noteworthy difference The mean plasma potassium level almost doubled—5 2 *to* 9 8 mEq/L—within 2 minutes after the onset of hyperventilation The changes in pH and pCO_2 were similar to the previous series

The ECG changes were more severe in this second group, and 9 of the 10 dogs developed ventricular tachycardia, one of which progressed into ventricular fibrillation This dog had the most profound and rapid rise in serum potassium—from 5 2 to 11 1 mEq/L in 3 minutes The epinephrine prevented posthypercapneic bradycardia and hypotension in these animals

DISCUSSION

This high incidence of severe cardiac arrhythmias sometimes leading to ventricular fibrillation has clinical and physiological significance

Clinical. It is important for the surgeon and anesthetist to realize that endogenous carbon dioxide can accumulate to dangerous *levels in short* periods of time—80 mm Hg in 15 min, 120 mm Hg in 30 min, and over 200 mm Hg in one hour The fact that rapid reversal of this hypercarbia results in precipitous changes in acid base balance and ionic equilibrium

which produce bradycardia, hypotension, grave cardiac arrhythmias and ventricular fibrillation, is of equal importance. Both carbon dioxide accumulation and rapid reversal of hypercarbia are preventable during surgery.

Hypercarbia during surgery results from inadequate elimination of CO_2 from the patient (ventilatory insufficiency), or inadequate elimination of CO_2 from the anesthesia apparatus (faulty rebreathing). When it does occur, unexplained tachycardia, tachypnea and rising blood pressure are early warning signs. Bradycardia, blood pressure depression and prolongation of the QRS complexes are later, more ominous signs.

Beecher[6] has emphasized the frequency and dangers of hypercarbia during clinical surgery. Dripps,[7] and Buckley, *et al.*,[8] have related this mechanism to the phenomenon of so called "cyclopropane shock." Although Sealy, Young and Harris[4] have shown experimentally that hypertonic glucose or saline can prevent these cardiac effects, prevention of the hypercarbia *remains the best clinical approach to the problem.*

We have recently learned of 2 surgical patients from elsewhere who developed fatal ventricular fibrillation on the basis of what appeared clinically to be rapid reversal of hypercarbia.

Case 1 J Z, a 58 year old white male with a history of angina and two previous coronary infarcts was taken to emergency surgery for resection of a hemorrhaging duodenal ulcer Numerous cardiac irregularities were noted throughout the operation,

defibrillation could not be maintained and he eventually succumbed.

Case 2 F R, a 41 year old white male who was normal except for a carcinoma of the rectum was taken to surgery for an abdominal perineal resection After 2 hours of surgery, the pulse rose to 130 within a 15 minute period At 4 hours, the pulse was still 130, respirations 50 and blood pressure 120/80 Faulty CO_2 absorption was suspected and the soda lime and anesthesia system were changed The new cannister became very warm when the patient was actively ventilated At the termination of the 4 hour procedure, while the perineal portion of the operation was being completed, the patient went into ventricular fibrillation Left thoracotomy, manual cardiac massage and serial defibrillation were effective in restoring a rhythmical heart beat However, following surgery, he failed to regain consciousness, developed seizures, hyperpyrexia, and progressive respiratory deterioration until he expired on the second postoperative day

Physiological. The profound speed with which marked changes in acid base balance and ionic equilibrium can occur in this situation are of absorbing interest. The initial transient hyperkalemia is probably due to a release of potassium from the liver on the basis of a stress reaction. The abrupt hyperkalemia at the time of CO_2 reversal can be explained by Fenn's report[9] that in the body, a decrease in CO_2 tension brings potassium out of the tissues. The simultaneous administration of epinephrine augments the hyperkalemia by its direct action on the sympatho adreno hepatic mechanism, as detailed by Houssay [10, 11]

The electrocardiographic effects of rapid reversal of hypercarbia are essentially the same as those noted with the rapid intravenous injection of KCl This is due to the sudden release of endogenous potassium stores. This relationship is further emphasized by the greater number and more serious cardiac arrhythmias noted in the second series where the plasma potassium was raised to higher levels by simultaneous epinephrine and

hypercarbia reversal A higher incidence of ventricular fibrillation was anticipated in this group of animals That this did not occur may have been due to a protective effect of epinephrine on the heart by preventing the usual post hypercapneic bradycardia and hypotension

The fact that ventricular fibrillation occurred in only 10% of our dogs as compared to higher percentages in the literature [1, 2, 3, 4, 5] is probably due to the shorter duration of severe hypercarbia exposure in these studies Sustained hypercarbia for 4 hours with 30 to 40% exogenous CO_2 also resulted in a sustained and progressive elevation of serum potassium in the work of Brown [3] Sealy,[4] and Young [5] Increased duration of hypercarbia and hyperkalemia tends to sensitize the myocardium and increase its excitability

Prasad Brown and Flink[12] have also shown that other ionic imbalances such as sudden decreases in ultrafilterable calcium may play a role in this phenomenon Certainly, other factors such as anoxia heart disease hemorrhage shock, trauma stress transfusions etc may operate to enhance these effects during clinical surgery In the final analysis however, severe cardiac arrhythmias and ventricular fibrillation resulting from rapid reversal of hypercarbia are attributable to potassium intoxication in the presence of other factors which operate to increase the sensitivity of the heart.

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THE RELATION OF THE SPECIFIC TISSUE TO THE COMMON MUSCLE IN THE HEART*

PETER A DEI MISSIER, ALFRED A ANGRIST, L CORSAN REID
AND J WILLIAM HINTON

The heart is composed of two different types of tissue. First, the specific tissue and second, the common myocardium. The specific tissue is phylogenetically the older structure and consists of the S A node, the A V node and the bundle of HIS and all its ramifications. It is the ultra *moriens* of the older writers and its is the last tissue in the body to die. In its main morphological expressions it differs markedly from the common myocardium, but its unique and outstanding feature is that it has the capacity to originate a stimulus autonomously. The common myocardium comprises the main mass of the heart and contracts only upon receipt of a stimulus normally from the specific tissue.

METHOD

1. The specific tissue was dissected in humans, dogs, pigs, sheep and steer, studied histologically, and compared to the common myocardium of the same animal.
2. Homogenates of specific tissue and common myocardium were compared in their reaction to various agents on their oxygen uptake as recorded by the usual manometric techniques.
3. In dogs on a heart bypass preparation the aorta was clamped and acetyl choline and potassium chloride injected in different animals to produce cardiac standstill.

RESULTS

The figures show a sharp histological difference between the common myocardium and the specific tissue. Twenty eight experiments on homogenates of common myocardium and 19 on the specific tissue exposed to *various agents will be summarized. Both are equally* depressed by malonate. The specific tissue is more sensitive to potassium iodoacetate than is the common myocardium. Nembutal does not depress oxygen uptake in either tissue. The specific tissue is much more depressed in its oxygen uptake by sodium pentothal and sodium amytal than the common myocardium. Methyl malonate enormously increases the oxygen uptake in the common myocardium and decreases the oxygen uptake of the specific tissue.

A movie picture clip shows upon injection of potassium chloride a sudden cardiac standstill which does not respond to any type of stimuli. Upon the injection of acetyl choline standstill also occurs, but now any type of stimulus applied to the common myocardium produces a contraction.

DISCUSSION

It is of some clinical significance that sodium pentothal and sodium amytal depress oxidative energy release much more in the specific tissue

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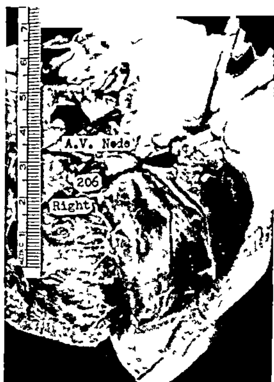


Fig. 1. Shows the A.V. node and the bundle of HIS with the right and left branches at their origin after removal of the interatrial septum.



Fig 2 Shows the left branch of the bundle of HIS breaking up into three main branches

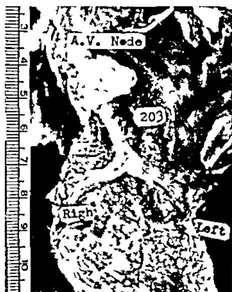


Fig 3 Shows the A V. node and the bundle of HIS with the right branch

than in the common myocardium, while nembutal has no depressing effect on either tissue The marked difference in the response to methyl malonate in these two tissues, as well as other evidence, strongly suggests that these two tissues are different metabolically as well as morphologically and functionally. The standstill that took place with potassium chloride injection is due to the action upon the common myocardium which is now unable to respond to any type of stimulus In the case of acetyl choline the stand-

still is due to the suppression of stimulus formation in the specific tissue and now any type of stimulus applied directly to the common myocardium produces a contraction

SUMMARY

Evidence has been presented to demonstrate the differences morphologically, functionally, and metabolically between the specific tissue and the common myocardium Cardiac standstill from the effects of potassium chloride is an effect upon the common myocardium preventing response to a stimulus, while with acetyl choline the effect is restricted to the specific tissue and now any stimulus applied to the common myocardium produces a contraction

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CARDIO PULMONARY TRANSPLANTATION*

WATTS R WEBB AND HECTOR S HOWARD

Successful transplantation of the heart and lungs requires the solution of many problems – technical, physiological and immunological The following series of experiments was performed for the technical development and physiological analysis of cardiopulmonary transplantations They embrace three different modalities (1) homologous transplants of the heart and both lungs, (2) autotransplants of the heart and heart lung preparation, and (3) transplantation of the heart combined with one lung

METHOD

Series 1. Cardiopulmonary Transplantation. Matched pairs of adult mongrel dogs were used with the combined heart and lungs of one being removed for re insertion into the second A sternal splitting incision was found to be the most satisfactory After the phrenic nerves were detached, the heart and lungs were dissected free except for major connections Inflow and outflow stasis was obtained and the heart perfused with lactated Ringer s solution to wash all blood from the coronary and pulmonary capillary beds [1] The trachea, aorta, and both cavae were then transected, and the heart and lungs removed as a unit Meanwhile, the recipient animal was prepared by a similar dissection and for maintenance with a pump oxygenator After removal of the recipient s own heart lung, that of the donor was inserted by coupling the inferior and superior cavae and careful suture anastomosis of the aorta Cardiac function was restored before anastomosis of the trachea, as respiration could be continued as long as desired by tracheal intubation

Series 2. Autotransplantation. In these experiments, the heart and lungs were dissected free from the mediastinum exactly as above The animal

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Supported by U S P H S Grant #H 2806

was heparinized with 1 mg heparin/kg body weight and placed on cardio pulmonary bypass The heart was not perfused but was allowed to continue beating during the inflow and outflow occlusion as suggested by Sen, Shah and Satoskar[2] Blood flows from the lungs through the left heart, to the aorta, through the coronaries, to the right heart and again to the lungs Thus, if the lungs are rhythmically inflated, there is oxygenated blood flowing through the coronaries during the entire period of isolation After all vessels were divided, the heart and lungs were lifted out of the chest and then replaced The cavae were anastomosed with couples and the aorta by direct suture The trachea was merely intubated until cardiac function had been restored and the anastomosis performed at leisure there after Seven preparations of this nature were performed, 4 autotransplant ing both the heart and the lungs and 3 transplanting only the heart In these latter, the pulmonary vessels were not divided and thus the heart was not completely removed at any time

Series 3. Homologous Heart and Unilateral Pulmonary Transplants. In this group, the heart, aorta and cavae were freed and the left stem bronchus and left lung isolated The phrenic nerves were detached from both sides of the heart The trachea and the right lung were undisturbed except for freeing the right pulmonary artery and veins These were left as long as possible for ease in coupling Much additional length can be obtained by the Sondergaard technique of dissecting the pulmonary veins, both superior and inferior, from the right atrium

The donor heart and left lung were perfused with lactated Ringer's solution, excised, and then refrigerated at 1°C in Tyrode s solution with 10% serum during preparation of the recipient animal[3] After the heart was chilled, couples were inserted in the cavae and the right pulmonary vessels The recipient animal was maintained by cardio pulmonary bypass while its heart and left lung were excised The donor heart and left lung were then inserted by suture anastomosis of the aorta and couplings of the cavae and right pulmonary artery and veins Cardiac action was restored prior to the bronchial anastomosis To prevent blood flow through the functionless left lung, the left pulmonary artery was clamped until the left bronchus was re anastomosed

OBSERVATIONS

Series 1. In 6 technically successful preparations of this nature, the donor hearts were out of circulation from 60 to 105 minutes The actual insertion of the heart in the later experiments required as little as 25 minutes In each of these preparations it was possible to restore the heart to relatively normal function and acceptable electrocardiographic patterns These animals lived from 75 minutes to 22 hours The early deaths were caused by continued oozing from the extensive raw surfaces developed during the dissection

A physiological impass occurred in this type of transplant in that these dogs with totally denervated lungs were unable to return to normal respira tion Even the dog which lived 22 hours before the experiment was abandoned never became able to breathe spontaneously

Series 2. The anastomoses required only 10 to 20 minutes before restora

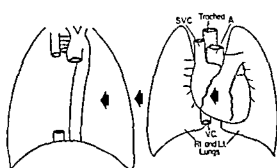


Fig 1 Diagram of anastomoses required for transplantation of heart combined with both lungs

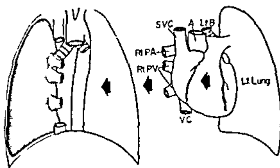


Fig 2 Diagram illustrating anastomoses required for transplanting heart with left lung

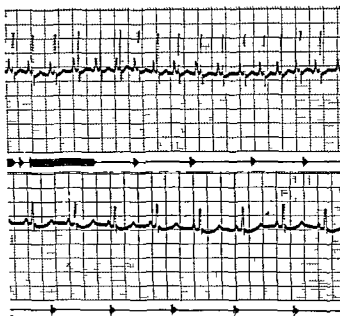


Fig 3 Electrocardiogram Top control Bottom Four hours post autotransplantation of heart and lungs Note slow rate from total denervation

tion of cardiac function In 2 of these preparations the aortic clamp was placed so close to the base of the heart as to occlude the coronaries These hearts fibrillated after 1 and 2 minutes of occlusion respectively and could not be resuscitated In each of the others the heart continued to beat slowly and easily during the entire procedure and immediately returned to satisfactory cardiac function with almost normal electrocardiograms After the cardiopulmonary autotransplants it was again observed that these animals were unable to breathe spontaneously although kept alive as long as 4 hours before being abandoned The animals with only their hearts transplanted and thus with relatively normal pulmonary innervation were able to return immediately to spontaneous respiration

Series 3 The donor hearts and lungs were exteriorized for 2 to 4 hours before function was restored It required approximately 30 minutes to anastomose the aorta and couple the five necessary vessels Again satisfactory cardiac function was easily restored in all but in one a twisted inferior vena cava caused complete obstruction and proved fatal before

it could be corrected Another died early from oozing The other dogs restarted spontaneous breathing as soon as the chest was closed and artificial respiration discontinued This technique proved undesirable in that the bronchial anastomosis behind the aorta was almost impossible through the anterior incision without displacing the heart and kinking the great vessels Transplantation of the heart and right lung may prove more desirable

DISCUSSION

Neptune and his associates[4] described transplantation of the combined heart and lungs in 3 dogs under hypothermia with one 6 hour survival Marcus Wong and Luisada[5] reported the use of interim perfusion of the donor heart while transplanting it and lungs into the abdominal cavity of dogs with anastomoses to the aorta and inferior vena cava This heart lung preparation was able to maintain life temporarily while the animal s own heart and lungs were excluded from circulation Our experience indicates that transplantation of the heart with both lungs will not be practical We have been unable in any of our various experiments to obtain restoration of normal respiratory function in the presence of total denervation of the lungs Comparable procedures will allow normal spontaneous breathing if the lungs have not been transplanted or if only one lung is transplanted While transplantation of the heart with one lung is more difficult the technique may be feasible for use in cases with pulmonary hypertension Whether or not the lung can be safely refrigerated is not settled Potts and his co workers[6] observed that lungs refrigerated overnight develop pulmonary edema when utilized as the oxygenator of an extracorporeal heart lung

These experiments indicate that the problems of technique and of physiology of cardiac transplantation can be solved As soon as the asso ciated immunological problems are solved cardiac transplantation should become a reality

CONCLUSION

1 Use of cardiac perfusion and refrigeration allows very adequate time for the performance of cardiac and cardio pulmonary transplantations For short periods of time autoperfusion of the coronaries by the isolated func tioning heart lung is a satisfactory method of maintaining the heart

2 Transplantation of the combined heart and lungs is technically easy but the denervation of the lung results in respiratory paralysis

3 Autotransplants of the combined heart and lungs lead to the same physiological impass

4 Transplantation of the heart with only one lung will circumvent the respiratory paralysis by leaving one innervated lung in the recipient

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TRANSPLANTATION OF THE HOMOLOGOUS HEART*

SALEM F SAYEGH OSCAR CREECH JR , AND JAMES H HARDING

Cervical transplantation of the homologous heart affords an opportunity to observe changes which affect survival of organ transplants This study was undertaken to determine the most suitable experimental animal for heart transplantation as a preliminary to investigating factors influencing survival This report concerns heart transplantation technique employed in dogs and rabbits and a comparison of survival of the grafts in these two species

METHOD

In this study transplantation of canine and rabbit hearts was performed Hearts were obtained from newborn puppies averaging 800 gm in weight and from fetuses removed by hysterotomy during the last 1 or 2 weeks of gestation Recipient dogs averaged 10 4 kg in weight Small rabbits averaging 1000 gm in weight were selected as donors while the recipients weighed about 3 4 kg

After anesthetizing donor and recipient with intravenous nembutal in a dose of 30 mg /kg electrocardiographic tracings were made of the donor heart and of the neck of the recipient to serve as control The technique of transplantation was essentially the same for the dog and the rabbit [1 2]

The external jugular vein and common carotid artery of the recipient were isolated and ligated Serrafine clamps were then applied to the proximal end of the vein and distal end of the artery and both vessels divided (Fig 1) The heart transplant was exposed through a bilateral thoracotomy incision and 5 0 mg of heparin injected into the inferior vena cava The thymus gland was dissected from the base of the heart the superior and inferior vena cavae in the dog plus the left superior vena cava in the rabbit were ligated and divided near the right atrium The ascending aorta and pulmonary artery were divided at the level of the transverse

*From the Department of Surgery Tulane University School of Medicine New Orleans Supported by National Heart Institute Grant HTS 5170 and the Louisiana Heart Association

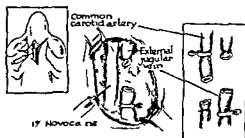


Fig 1 Technique of Transplantation The common carotid artery and the external jugular vein in the recipient are isolated the adventitia of these vessels infiltrated with 1% novocaine The distal end of the vein and the proximal end of the artery ligated and the vessels divided

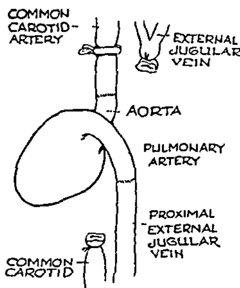


Fig 2 Technique of Transplantation The aorta of the graft is anastomosed to the distal end of the common carotid artery, and the pulmonary artery to the proximal end of the external jugular vein

sinus The lungs were then removed after the pulmonary veins were ligated and divided

Arterial silk 6 0 or 7 0 was used for anastomosing the ascending aorta and the pulmonary artery of the graft to the distal end of the common carotid artery and the proximal end of the external jugular vein of the recipient (Fig 2)

The period of cardiac standstill varied between 20 to 30 minutes In some instances the heart continued to contract feebly throughout the procedure

Table 1 Comparison of Results of Cervical Transplantations of the Rabbit and Canine Hearts

RECIPIENT	NUMBER OF EXPERIMENTS	AVERAGE SURVIVAL (HOURS)	AVERAGE WEIGHT (KILOGRAMS)
Rabbits	11	130 (6 576)	3 45
Dogs			
Fetus	10	73.2 (12 201)	
			10 4
Puppy	21	81 (12 210)	

Cardiac contractions started promptly after releasing the occluding clamp on the carotid artery In three fourths of canine heart transplants the initial cardiac action was ventricular fibrillation which reverted spontaneously to a normal sinus rhythm within seconds No fibrillation was noticed in rabbit heart transplants

RESULTS

Thirty-one canine and 11 rabbit hearts were transplanted In the dog, fetal hearts survived (Table 1) from 12 to 204 hours with an average of 73 2 hours, whereas the survival of puppy hearts ranged from 12 to 240 hours with an average of 81 hours In the rabbit, survival of the transplants ranged from 6 to 576 hours with an average of 130 hours

There were three patterns of failure of the grafts as determined by electrocardiographic studies and these were related to the period of survival

1. **Ischemic Failure** was observed in grafts surviving less than 72 hours and indicated acute failure due to thrombosis of the vessels and infarction
2. **Mechanismal Failure** was due to the progressive changes in rhythm and was observed in grafts surviving longer periods
3. **Reactive Failure** was observed in grafts surviving more than 1 week and was characterized by progressive diminution of the QRS amplitude indicating myocardial degeneration

Pathologic changes observed in the grafts depended on the length of survival Grossly there was little or no change in grafts that failed within 24 hours except for thrombosis of the aorta or pulmonary vein or distention of the graft with blood Grafts surviving longer periods were surrounded by a layer of fibrin with evidence of organization in those transplants surviving more than one week Discoloration and softening was observed in grafts surviving more than 7 days

The microscopic appearance varied with the type of failure and the survival period Thus, early failures were characterized by slight infiltrations of erythrocytes between the myocardial fibres Progressive degeneration of the myocardium with round cell infiltrations was observed in grafts surviving longer periods

SUMMARY

1 Homologous transplantation of the heart provides a valuable method for the study of factors influencing survival of homologously transplanted organs

2 The electrocardiographic and pathologic changes in the grafts depend on survival of the graft and the pattern of its failure

3 Survival of grafts was longer in rabbits than in dogs particularly if the recipients were large

4 Maximum survival of a homologously transplanted rabbit heart of 576 hours is recorded

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Heart

B Coronary Physiology, Asystole and Valvular Disorders in the Heart

EVALUATION OF CONTRAST MEDIA EMPLOYED FOR AORTIC AND CORONARY VISUALIZATION*

OTTHEINRICH HASE AND RALPH A DETERLING, JR

The expanding field of roentgenographic techniques with opacification of the cardiovascular structures has further stimulated the search for safe and useful contrast substances Although the newer contrast media of the tri iodinated aminobenzoic acid series (Urokon®, Hypaque®, Miokon®, Renografin®) have already found wide clinical use throughout the country a certain number of side reactions incident to their use still do occur An experimental study of their cardiovascular effects was conducted similar to earlier work in this laboratory in which the older media (sodium iodide, Thorotrast, Diodrast Neo Iopax) were tested[1]

METHOD

Aortic Injection Mongrel dogs weighing 6 to 20 kg were anesthetized with Nembutal 30 mg /kg and respiration was controlled with a mechanical respirator delivering oxygen Aortic and peripheral arterial pressures were recorded through polyethylene catheters and Statham strain gauges by means of a multiple channel oscillograph † Lead II of the electrocardio gram was recorded Continuous slow infusions of 5% dextrose or isotonic sodium chloride solution with small amounts of heparin were administered through the catheters in order to keep them patent Injections of the contrast media were performed into several areas of the thoracic aorta through end holed polyethylene catheters introduced peripherally Catheter sizes of 0 045 to 0 066 inch internal diameter were used and fitted to #15 to 18 standard needles Injections were done as rapidly as possible by hand with a 20 ml glass syringe

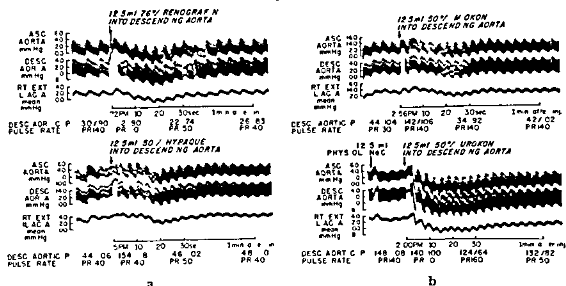
RESULTS

As a representative example, the effect of an intra aortic injection of each of the four media tested is shown in Figure 1 In order to elicit pharmacological differences the media have been compared on a ml /kg body weight basis The characteristics of the reaction were similar in all four instances They were comparable to those described for the older media and especially exhibited by sodium iodide A few hypertensive beats incident to the sudden injection initiate a short phase of systolic and diastolic pressure elevation After 5 to 10 seconds a hypotensive phase

†Manufactured by Electronics for Medicine White Plains N Y

*From the Department of Surgery College of Physicians and Surgeons Columbia University New York Supported by grants from the New York Heart Association Supported in part by U S P H S Grant #H2506

Fig 1



a b

Fig 1 Experiment 21 Dog 593 B — Record of the cardiovascular effects of different contrast substances injected into the descending aorta of a dog of 12 4 kg weight These four records have been selected out of a series of 12 injections into one animal The order is indicated by the time The four preceding and the four following injections showed the same relative effect — Nembutal anesthesia

a — Renografin 76%
Hypaque 50%

b — Miokon 50%
Urokon 50%

follows, often with a widening of the pulse pressure and tachycardia More violent immediate reactions are characterized by short periods of asystole, nodal rhythm and bradycardia for the first 5 to 10 seconds after injection Within 30 seconds, or after 1 to 2 minutes, the blood pressure fluctuation has usually subsided and the values have become stabilized at the pre injection level, or slightly lower Even the most drastic reactions, *such as produced by sodium iodide, have disappeared after 10 to 15 minutes*

The frequently observed periods of asystole and bradycardia immediately after injection occurred irrespective of the site of the injection within the thoracic aorta and appear to be a peripheral vascular reflex These ar rhythmias and the following hypotension also persist after bilateral excision of the carotid sinus or section of the vagus nerves If the substance was injected into the inferior vena cava, however, the reaction was much milder

No fatal incidents occurred which could be attributed to a single or even to repeated injections of the media The general tolerability was good for as many as 10 or 12 different injections of 10 ml each during a prolonged experiment Continuous hydration with dextrose and saline infusions, and minimal doses of the anesthetic agent were believed im portant in the reduction of toxicity A marked diuresis was noted in prolonged experiments

As has also been noted by Rowe and associates,[2] the degree of reaction to any of the media is to some extent an individual matter and may vary from animal to animal The ml /kg of body weight ratio *per se* is not useful if dogs are of considerably different size Five milliliters injected into a dog of 6 kg, for example, will usually produce less effect than

systoles appeared incident to the injection Hypaque 90% and Miokon 90% *tested in this fashion did not produce* greater alterations than their 50% solutions Injections of drugs like adrenalin or Isuprel, or potassium or calcium did produce the immediate characteristic effects of these agents

Visualization study. Aiming at a practical method by which the visualization qualities of the various media could be tested with a reasonable reproducibility in the living animal, a technique was developed which permits injection of small quantities of contrast medium into the ascending aorta during a precise fractional period of the cardiac cycle For this purpose, a pressure injection device was constructed with delivery up to 50 ml per second (less for high viscosity media) and operated electrically Using the R wave of the animals electrocardiogram as a pacemaker, and by means of amplifying and time delay systems,† it is possible to inject at any desired moment of the cardiac cycle Variable amounts of contrast substance can be delivered within a chosen time The radiographic exposure is triggered electrically over a separate adjustable delay Both injection and roentgenographic exposure can be operated automatically for consecutive cardiac cycles Marks of injection and x ray exposure appear on the oscillographic cardiovascular record Coronary visualization obtained with this technique is shown in Figure 2

The delivery *of comparatively small amounts of contrast medium* during specific fractional periods of the cardiac cycle may reveal information on the fluid dynamics of coronary arterial filling Contrast substance then correctly injected at periods favorable for coronary inflow would make *possible good roetgenographic visualization with a minimum of contrast* medium Further experimentation with this method is in progress

SUMMARY AND CONCLUSIONS

1 Urokon, Hypaque, Miokon, and Renografin have been tested in the anesthetized dog Arrhythmias and blood pressure fluctuations can be produced with all four substances Compared in 50% concentrations Hypaque and Miokon appear most suitable for aortography

2 Injection of the media directly into the coronary arterial bed produces transient T wave changes and often prolongations of the diastolic interval From a comparison of the effects on heart rate and blood pressure, Hypaque 50% and Miokon 50% have been selected for coronary angiographic studies

3 As a practical approach to coronary visualization, an electrically operated pressure injector was constructed which permits injection of small amounts of contrast medium rapidly, during a precise fractional period of the cardiac cycle, and electrical time control of the radiographic exposure

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†Electrical circuits constructed by Dr Duncan A Holaday

DOES INTERNAL MAMMARY LIGATION INCREASE ARTERIAL FLOW TO THE MYOCARDIUM?*

JAMES C GRIFFIN, JR, JAMES D HARDY, AND M D TURNER

Internal mammary artery ligation is one of the more recent procedures employed with the hope of increasing the blood flow to the myocardium It has been claimed that a significant number of patients with angina pectoris are improved by this operation The rationale involved is that bilateral ligation of the internal mammary artery in the second or third interspace results in additional flow through small vessels that arise from the internal mammary proximal to the point of ligation

The purpose of the present study was to learn whether or not ligation of the internal mammary artery in dogs could produce increased arterial flow to the myocardium

METHOD

Fifty two mongrel dogs of both sexes ranging in weight from 6 8 to 11 1 kg were used All operations were performed under intravenous nembutal anesthesia The experiments were as follows

Part I. Demonstration of Vascular Channels From the Internal Mammary Artery to the Pericardium In 5 dogs the chest was entered through a sternal splitting incision The internal mammary arteries were cannulated with polyethylene catheters that were threaded into the subclavian artery and into the internal mammary artery A ligature was placed around the internal mammary artery in the third intercostal space, and methylene blue was then injected into this artery The dye was seen to pass readily downward through many fine vessels which coursed to the pericardium and even to the epicardium overlying the auricles

Thus since vascular connections between the internal mammary artery and the pericardium had been demonstrated, the next step was to determine whether or not this blood from the internal mammary artery actually entered the myocardium to return to the right atrium by way of the coronary sinus

Part II. Experiments to Determine the Presence or Absence of Flow in Pericardial Vessels from Internal Mammary Artery to Coronary Sinus. *A Search for Blue Dye in Coronary Sinus Blood* Attempts to demonstrate the presence of blue dye in the coronary sinus blood, derived directly from the dye injected into the internal mammary artery, were inconclusive because of the rapid recirculation of blood in the dog When a catheter was placed in the coronary sinus for blood sampling, from 8 to 10 seconds were required for the slow venous stream to propel the blood through the catheter Therefore, since the average circulation time of the dog is only from 6 to 9 seconds, it could not be stated unequivocally that dye which reached the coronary sinus catheter might not have been derived from recirculation of the dye injected into the internal mammary artery

*From the Department of Surgery University of Mississippi Medical Center, Jackson
Aided by a grant from the Mississippi Heart Association

B. Detection of Injected I^{131} Albumin in Coronary Sinus Blood. I^{131} tagged albumin was injected into the internal mammary artery and an attempt made to stop the heart with an electric defibrillator within 3 seconds, before recirculation could have occurred. This was inconclusive for two reasons: (1) the heart could not be stopped consistently at the desired moment, (2) when the pericardium was incised to check the heart for the presence of the isotope, contamination occurred.

C. Effect of Potassium Chloride Upon Myocardial Rate Following Injection Into the Internal Mammary Artery. In 12 dogs the pericardium was opened and the ascending aorta dissected out. A clamp was placed across the aorta just proximal to the innominate artery. A saturated solution of KCl varying from 0.1 to 0.25 cc. was injected into the left ventricle immediately following clamping of the aorta. Whereas no serious effect upon heart rate occurred if the aorta was not clamped, occlusion of the ascending aorta just prior to injection of 0.25 cc. of saturated KCl into the left ventricle resulted in cardiac standstill or fibrillation in 100% of the animals; 0.1 cc. of saturated KCl produced cardiac arrest in 75% of the dogs so treated.

In contrast to the injection of 0.25 cc. of KCl into the left ventricle (with aortic occlusion) which produced 100% mortality, the injection of even 2.0 cc. of KCl into the internal mammary artery of 16 dogs whose internal mammary arteries had not previously been ligated produced tachycardia with stronger contractions; however, cardiac arrests were then produced by injection of 0.25 cc. of KCl into the ventricle following aortic occlusion.

Six dogs that had had previous *right* internal mammary ligation showed no evidence of cardiac standstill upon injection of KCl into the internal mammary. If 2.0 cc. of KCl, injected into the internal mammary artery failed to produce cardiac standstill, then 0.1 cc. of KCl was injected into the left ventricle while the aorta was momentarily clamped. Cardiac arrest occurred in all animals with left or right internal mammary ligation immediately after injection of the KCl into the ventricle. Out of 8 dogs whose *left* internal mammary artery had been previously ligated, 4 exhibited prompt cardiac arrest upon the injection of 2.0 cc. of saturated KCl solution into the left internal mammary artery. This arrest occurred so quickly that the possibility of recirculation of KCl as a causative factor was virtually excluded. The pericardium was opened in these 4 animals which died following internal mammary artery injection, and in 2 animals gross discoloration of the blood contained in the coronary arteries was observed. This finding, along with the instantaneous cardiac arrest following KCl injection into the left internal mammary after prolonged ligation, clearly indicates communication between the internal mammary artery and the myocardium in the 4 animals mentioned above. Methylene blue was used

DISCUSSION

Injection of 0.25 cc. of a saturated solution of KCl directly into the chamber of the left ventricle with momentary clamping of the ascending aorta resulted in cardiac arrest in 100% of all normal dogs in this series. The aorta was clamped at the same instant of injection of the KCl solution in order to allow a high concentration of potassium to perfuse the coronary

vessels Cardiac arrest was produced in about 75% of normal dogs attempted by the injection of 0 1 cc of saturated KCl into the left ventricle Since these relatively small amounts of potassium caused cardiac standstill under the above conditions it was believed that an injection of larger amounts of potassium into the internal mammary circuit would result in cardiac arrest if the vascular communications were present in significant amounts However, no arrests occurred following this procedure except in 4 of 8 animals in which the *left* internal mammary artery had been ligated for prolonged periods No arrests occurred in normal animals or in animals with right artery ligation Bilateral ligation of the internal mammaries has not been studied by the above procedure

We are of the opinion that in a small number of dogs communications are developed between the pericardial arterioles and the coronary arteries We can only theorize that these communications most likely occur at the point where the small branches from the coronaries supply the base of the aorta and pulmonary artery and the reflexion of the pericardium over these major vessels

Although some anastomoses apparently develop, we believe that only a minute amount of blood flows into the coronaries from the pericardium It definitely is not clear whether or not this blood flow represents a significant amount in the maintenance of an ischemic myocardium

SUMMARY AND CONCLUSIONS

Using 52 dogs, an attempt has been made to demonstrate blood flow directly from the internal mammary artery to the venous drainage of the myocardium The injection of methylene blue dye, I^{131} tagged albumin, and saturated KCl solution demonstrated numerous vessels which course from the internal mammary artery to the pericardium and epicardium However, it has been much more difficult to establish the presence of blood flow from the numerous pericardial vessels to the drainage system of the myocardium It appears that left internal mammary ligation resulted, in several weeks or months, in some slight increase in blood flow to the myocardium in a few of the animals studied

CORONARY ARTERIOGRAPHY IN THE ADULT HUMAN PATIENT*

ALAN P THAL, L STEPHEN RICHARDS AND M JOHN MURRAY

During the performance of retrograde aortography the coronary arteries have been identified incidentally in the occasional patient. The most complete radiographic study of the coronary arteries in the human has been made by Di Guglielmo[1] who reported on 192 arteriograms. These arteriograms were made chiefly during retrograde aortography in children with congenital heart disease and all these studies were performed under general anesthesia. A precise knowledge of the anatomic pathology of the coronary arteries during life would add considerably to knowledge of the evolution of coronary sclerosis and might also offer a sound basis for surgical intervention.

The present paper serves as an interim report on the technique we have used in performing coronary arteriograms on 20 adult patients.

METHOD

While our initial coronary arteriograms were done under general anesthesia in patients receiving intra aortic nitrogen mustard for metastatic malignancy, all subsequent studies have been made under local anesthesia with minimal sedation. The age range of these 20 patients has been from 17 to 76 years. About 30 minutes prior to the injection of the contrast material the patients were given 50 mg of dramamine and 25 mg of phenargen intravenously and about 10 minutes before the procedure 1/100 grain of atropine was given intravenously. After infiltrating the medial aspect of the upper arm with procaine, the brachial artery was exposed and dissected free for a distance of about 3 cm. It was secured both proximally and distally by means of bulldog clamps and a 1 cm longitudinal arteriotomy was made. A #10 French thin walled Lehman cardiac catheter was then threaded through the brachial artery. Under fluoroscopic control, using the image intensifier, the catheter was gently maneuvered into the ascending aorta. The majority of our cases have been approached from the right side. In 3 instances we were unable to rotate the catheter into the ascending aorta. Subsequently, 2 patients were catheterized through the left brachial artery. It is our present impression that cannulization from the left side gives better access to the ascending aorta. Experience in both the dog and the human has shown that the optimum position of the tip of the catheter is about 5 to 7 cm above the sinuses of Valsalva. With the catheter tip situated further away from the coronary orifices too great dilution takes place and when it is positioned at the level of the coronary orifices one or other of the coronary vessels may fail to fill with the radiopaque material. Several contrast materials have been

*From the Departments of Surgery, Radiology and Medicine, The University of Minnesota Medical School, Minneapolis. Supported by grants from the United States Public Health Service, The American Heart Association, and the Minnesota division of the American Heart Association.

tried both in the dog and in the human In the last 11 cases Renografin 76% has been used and has thus far proved entirely satisfactory

Injection Devices In our original studies a simple injection block previously described[2] was used for rapid injection of dye but this has been entirely superceded by a pressure injection stand of the simple lever type With this device 40 cc of 76% Renografin may easily be injected in 1½ seconds

The Rapid Film Changer And Exposure Factors Our initial x rays were taken on the Rigler Watson rapid film changer Five exposures per second were made using 400 or 500 milliamperes and 95 kilovolts at one thirtieth or one sixtieth of a second At the present time studies are being made on the Schonander biplane machine This promises to give superior results

The carotid arteries are compressed bilaterally during the injection to diminish cerebral circulation of the radiopaque material Following the injection the patient may experience a slight sensation of warmth Aside from this there have been no reactions of any consequence to the injection of contrast material Immediately after the injection the catheter is withdrawn The arteriotomy is irrigated with saline and precisely closed with 6 0 arterial silk

In two instances cinephoto fluorograms have been taken to illustrate actual circulation of dye However these studies on 16 mm film are not sufficiently detailed to be of diagnostic value

With accurate placement of the catheter and rapid injection of dye it has been possible to consistently obtain detailed coronary arteriograms Studies have been made in a number of disease states including hypertensive cardiovascular disease senile arteriosclerosis and severe angina pectoris A feature of the present study has been the absence of any sequelae in patients with severe occlusive coronary disease Detailed arteriograms on these patients have been published elsewhere[2]

Demonstration of Cardio Pulmonary Anastomotic Channels Following Pulmonary Artery Occlusion Studies by Liebow in the dog have shown that large collateral vessels develop between the coronary arteries and the bronchial arteries after occlusion of a main pulmonary artery The following case demonstrates the expansion of these collaterals in the human following pneumonectomy

Coronary arteriography was performed on a 55 year old man who underwent pneumonectomy for carcinoma of the left lung in 1954 Subsequently he developed intermittent episodes of chest pain not clearly related to effort The electrocardiogram was interpreted as being compatible with myocardial ischemia because a left fibrothorax coronary arteriogram was performed in a lateral position This is illustrated in Figure 2 Serial studies of the coronary arteries showed no detectible lesion However a large branch ascending from the right main coronary artery to the anterior mediastinum was regularly seen in serial studies This vessel was not identified in coronary arteriograms of other patients and is thought to represent an expanded cardiopulmonary collateral vessel resulting from occlusion of the pulmonary artery

Electrocardiographic Changes During Injection All patients were carefully monitored on an electrocardiograph before during and after coronary arteriogram No ischemic changes were demonstrable There was slight bradycardia during the injection of contrast material This in all probability is the result of carotid compression



Fig 1 Coronary arteriogram in a 43 year old male with angina decubitus Blood flow to this heart was almost entirely by way of a sclerotic but unoccluded right coronary artery seen in this figure The left main coronary artery was occluded A very similar picture has recently been seen in a 32 year old male with angina decubitus (By permission of Surgery Gynecology and Obstetrics)



Fig 2 Coronary arteriogram in the lateral projection showing a collateral vessel between the right main coronary artery and vessels in the anterior mediastinum As described in the text, here is reason to believe that this vessel is an expanded cardiopulmonary collateral (By permission of Surgery Gynecology and Obstetrics)

COMMENT

The technique used for coronary arteriography is simple and reproducible The quality of the arteriograms obtained are sufficiently detailed to be of value diagnostically Our initial experiences with biplane coronary arteriography, not reported here, suggest that this method will give a more detailed picture of the lesions affecting the coronary arteries It seems probable that this technique will yield considerable information about the natural history of coronary artery disease It has already, in our hands, proved useful in resolving difficult clinical diagnostic problems A striking feature of this study has been the tolerance of patients with advanced occlusive coronary artery disease to the retrograde injection of the contrast material into the arch of the aorta

SUMMARY

A technique is described for coronary arteriography under local anesthesia in the adult human patient Detailed coronary arteriograms have

been regularly secured and the procedure has been well tolerated in all instances thus far

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AN ARTIFICIAL CONDUCTION SYSTEM FOR THE MANAGEMENT OF EXPERIMENTAL COMPLETE HEART BLOCK*

M JUDAH FOLKMAN, AND ELTON WATKINS

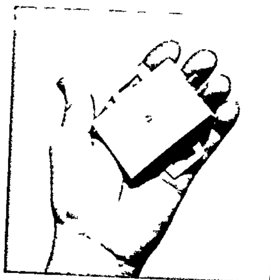
In complete heart block it would be desirable to return ventricular contraction to the control of the sino atrial node so that autonomic regulation of heart rate could be re established Such a method would have merit in helping patients through the stresses of postoperative recovery after they had sustained conduction system injury during intracardiac surgery To achieve this type of conduction control we have constructed a miniature transistor amplifier which amplifies the auricular impulse potential enough to cause contraction of the ventricles by direct electrical stimulation

METHOD

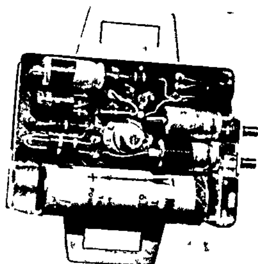
Creation of Complete Heart Block. Complete heart block was created in 24 dogs by modification of a method described by Starzyl[1] A right fourth interspace incision gives the best exposure During a 1 minute period of complete venous occlusion, the right atrium is opened and the Bundle of His is encircled with a #0 atraumatic silk suture placed in the auricular septum 1 cm anterior to the coronary sinus The suture passes at right angles to the tricuspid valve ring encompassing a generous amount of atrial septum and tricuspid valve annulus at the base of the septal cusp Complete heart block occurs as the suture is snugged down Four of the dogs were used in acute experiments Nineteen of the remaining 20 dogs have survived with complete heart block during periods of observation up to 1 year During the postoperative period, ephedrine was occasionally required to prevent cardiovascular collapse

Development of a Transistor Amplifier We found by cathode ray oscilloscope measurement that the average contraction potential recorded directly from the right atrial surface was 10 millivolts, in a dog with complete heart block An impulse of 450 millivolts from an electric stimulator was needed to actuate ventricular contraction when electrodes 1 sq cm in area were placed on the ventricular surface

*From the Surgical Research Laboratory of The Children s Medical Center of Boston and The Harvard Medical School Boston Supported by a grant from the National Heart Institute of the U S Public Health Service and by the American Heart Association



A



B



C

Fig 1 Transistor Amplifier (A) Transistor amplifier with gain control and input and [illegible] amplifier [illegible]og s har[illegible] battery in place (C) Amplifier attached to dog in which complete heart block had been previously produced Heavy bandaging is needed to prevent animal from shaking loose the amplifier

Dr Frederick Vanderschmidt, of the Massachusetts Institute of Technology, helped build a small transistor amplifier which would increase the 10 millivolt auricular impulse to the 450 millivolt potential we found necessary to initiate ventricular contraction This amplifier weighs less than 2 oz, fits into the palm of one's hand, and is powered by tiny batteries which last about one month The amplifier has a variable gain control to alter the voltage output This allows adjustment to the minimal voltage necessary for ventricular activation A socket is provided for plugging in a temporary auxiliary battery pack, so that the tiny batteries may be changed without interrupting amplifier function and normal conduction The amplifier is easily carried on the shoulder harness of a dog so that the animal may move about without restraint (Fig 1)

Electronic Correction of Complete Heart Block. Dogs with surgically induced heart block were subjected to another thoracotomy through the

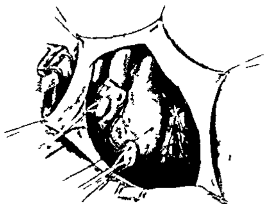


Fig 2 Technique of Attaching Electrodes to the Heart Stainless steel electrodes with polyethylene supports are sutured to the right auricle and the right ventricle with #3 0 silk sutures

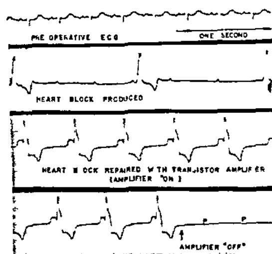


Fig 3 Electrocardiographic Tracings With Amplifier in Operation When the amplifier is in operation the complete heart block is converted to a normal electrocardiographic pattern What appears to be the short P R interval of the Wolff Parkinson White syndrome may actually be the result of the very fast conduction in this electrical system as compared to the conduction through the Bundle of His

right fourth interspace Two small (less than 1 sq cm in area) stainless steel electrodes sealed to a polyethylene support, were sutured to the right auricle Two additional electrodes were sutured to the right ventricle (Fig 2) Leads insulated with fine polyethylene tubes were then passed to the skin surface through a stab wound, and attached to the transistor amplifier The auricular electrodes pick up impulses from the atrial surface and transmit them to the transistor apparatus These impulses are amplified and fired back into the ventricular myocardium through the ventricular electrodes, thereby converting the complete heart block to a normal sinus rhythm

RESULTS

When the amplifier is functioning the electrocardiogram shows a normal electrical pattern The heart rate ranges between 90 and 130/min depending upon the animal s activity The dog is frisky and eats well When the amplifier is turned off the electrocardiogram reverts to a complete block, the pulse rate drops to about 30 beats/min, and occasionally the animal collapses in a Stokes Adams attack (Figure 3)

The amplifier has been observed during continuous operation in 5 dogs up to periods of 3 weeks Each animal s circulatory status has been improved by the electronic device In another 5 dogs, the amplifier has been

used intermittently to observe its performance during exercise and in the presence of auricular fibrillation

Auricular fibrillation occurred in one of our dogs, while the amplifier was functioning This produced a ventricular tachycardia Consequently we are now designing an amplifier with a choking circuit which will prevent the ventricles from receiving impulses at a rate over 130/min Any other rate may be selected as a maximum The circuit will produce a regular ventricular beat in the presence of auricular fibrillation

We do not know what happens to the myocardium under the electrodes after long term stimulation for several months Under such conditions, enough fibrosis might occur to prevent electrical stimulation of the ventricle This aspect is under investigation The durations of successful artificial conduction studied seemed sufficient to warrant clinical trial of the apparatus in the short term block sometimes seen after ventricular septal defect repair An opportunity to do this has not occurred It is quite possible that if auriculo ventricular block has been produced in a human while operating upon an interventricular septal defect, support of the patient for 2 or 3 weeks by such an electronic device as described here would allow sufficient time for the patient to regain use of normal function of his own conduction system

SUMMARY

A method of artificial electronic correction of complete heart block in experimental animals has been described A smaller transistor amplifier has been used to magnify auricular electrical impulses and transmit them directly to the ventricles, which are thereby stimulated This device has been tried in *10 dogs with complete heart block, with beneficial result* in all animals The possibility of clinical application is discussed

The authors wish to express their indebtedness to Dr Robert F Gross for his continued encouragement and kind direction in this study and to Dr Frederick Vanderschmidt for his invaluable contribution to the design of the transistor amplifier

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EXPERIMENTAL CORONARY ARTERY OCCLUSION VENTRICULAR FIBRILLATION AND SURVIVAL AS AFFECTED BY SELECTED DRUGS AND IONIC ALTERATIONS*

WILLIAM T WILLIAMS, ALBERT L MEENA, M DON TURNER, AND JAMES D HARDY

This report concerns experimental myocardial ischemia, produced by acute coronary occlusion and the influence of drugs and inorganic ions on survival rates and early fibrillation

METHOD

Adult mongrel dogs were used as the experimental animals Following endotracheal intubation, the chest and pericardium were opened through the usual incisions and the left coronary, circumflex coronary and left anterior descending coronary arteries were carefully demonstrated A heavy silk ligature was placed beneath the anterior descending branch imme diately adjacent to its origin The loose ligature was threaded through a small plastic tube which was anchored to the pericardium at one end and brought out through the chest wall at the other end Following closure of the chest wall, the ligature ends and plastic tube were placed sub cutaneously

The following day the animal was again anesthetized, control electro cardiograms obtained and control blood samples drawn for plasma pH, potassium, sodium, chloride, calcium and carbon dioxide tension The animals were then divided into the following groups, depending upon the ion or drug which was administered (1) control, 1 day postoperative, 10 dogs no infusion, (2) control, 20 days postoperative, 3 dogs, no infusion, (3) acidosis, 5 dogs 100 to 140 mEq hydrochloric acid in 400 cc water, (4) alkalosis 5 dogs, 140 to 150 mEq sodium bicarbonate in 400 cc water, (5) hyperkaliemia, 5 dogs 30 to 45 mEq potassium chloride in 400 cc water, (6) hypercalcemia, 5 dogs, 1 gm calcium chloride in 400 cc 5% dextrose in water, (7) excess sodium chloride, 5 dogs, 4½ gm sodium chlo ride in 500 cc distilled water, (8) procaine, 5 dogs, 500 mg "Novacaine" in 400 cc 5% dextrose in water (9) papaverine, 5 dogs, 30 mg papaverine intravenously, (10) quinidine, 5 dogs, 100 mg quinidine intravenously, (11) growth hormone, 5 dogs, growth hormone 300 mg intramuscularly (12) growth hormone, 5 dogs, growth hormone 200 mg intramuscularly

Immediately following the infusion, electrocardiograms were again ob tained and blood samples drawn for the determinations listed above The ligature was tightened completely occluding the left anterior descending coronary artery, and electrocardiograms were obtained at frequent intervals to one hour postocclusion Early fibrillation as used in this communication denotes fibrillation occurring during the 60 minutes following coronary artery occlusion Mortality was determined at the end of the first 24 post occlusion hours

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*From the Surgical Experimental Laboratory and the Department of Surgery, University of Mississippi School of Medicine *Jackson* Supported by U S Public Health Service Grant No H 2806 and Mississippi Heart Association Grant

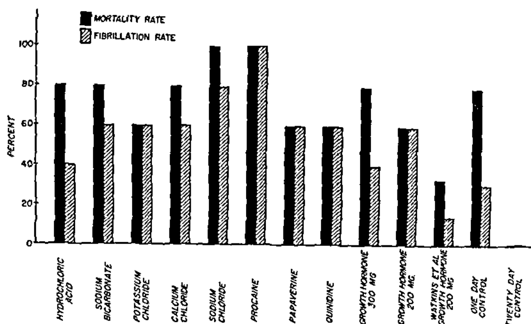


Fig 1

RESULTS

The mortality and fibrillation rates of the various groups are shown in Figure 1

DISCUSSION

(1) **Control.** One day postoperatively, mortality rate 80%, fibrillation rate 30% These results are in agreement with other reported series of coronary artery occlusion of the left anterior descending branch

(2) **Control.** Twenty postoperatively, 0% mortality, 0% fibrillation This experiment was designed originally to allow the animal to recover from thoracotomy and dissection around the coronary artery It was found *that if ligation were delayed following pericardotomy, we failed to achieve* any mortality This point was borne out in 3 cases in which ligation was delayed for 20 days after thoracotomy, in which there was no mortality *in the 3 animals and in which 2 of the 3 failed to show any change in* the postocclusion electrocardiogram

(3) **Hyperkaliemia.** Mortality rate 60%, fibrillation 60% The mean potassium level was elevated to 6 24 mEq /L from a mean control of 3 7 mEq /L by infusion of potassium chloride Recent investigation has strongly implicated potassium as the excitatory agent for ventricular fibrillation Harris *et al*, following the intracoronary infusion of potassium chloride, noticed that the intensity of ectopic ventricular activity depended upon the amount of potassium chloride present in the coronary arteries of dogs Following coronary ligation, there was a large increase in the potassium content of venous blood from the ischemic area, increasing from a control level of 12 75 mg % to 21 5 mg % The potassium concentration showed a positive correlation with the ectopic activity In addition, potassium concentration reapproached control levels during the periods that corresponded to the times of disappearance of ectopic activity At that

time potassium content of the infarcted muscle was greatly reduced Montgomery Prevedel and Swan likewise obtained convincing evidence relating potassium and plasma pH to ventricular fibrillation Hooker found that ventricular fibrillation in the isolated perfused heart could be converted by the addition of potassium to the perfusing medium Our studies suggest that there is no correlation between serum potassium level and ventricular fibrillation When the serum potassium was at its lowest level as in the sodium bicarbonate and calcium chloride series a fibrillation rate of 60% occurred When the serum potassium was considerably increased by the infusion of potassium chloride fibrillation again occurred in 60% of the animals Thus it would appear that if potassium is the excitatory agent for ventricular fibrillation myocardial potassium is unaffected by plasma concentration and is on a metabolic basis

(4) **Acidosis** Mortality 80% fibrillation 40% Hydrochloric acid in the amounts used in this study produced an acidosis with a mean pH of 7 22 as opposed to a mean control pH of 7 37 Montgomery s observation showed that blood pH influenced the concentration of potassium in the myocardium demonstrating that with a low pH the heart takes up potassium whereas with a high pH the heart maintains potassium balance In our series the mortality and fibrillation rates in the acidosis group most closely approached the control levels whereas the alkalosis group demonstrated fibrillation rates twice the control levels although the ultimate mortality was the same Again if potassium is the agent inciting ventricular fibrillation it would appear that myocardial metabolism of potassium in myocardial ischemia is unaffected by blood pH insofar as decreasing early fibrillation is concerned

(5) **Alkalosis** Mortality 80% fibrillation 60% The mean pH was elevated to 7 62 from a mean control of 7 37 by the infusion of sodium bicarbonate Bellet in discussing the possible modes of action of 0 5 molar sodium lactate in preventing or correcting cardiac arrhythmias considered that one of the beneficial actions of this solution might be due to the production of alkalosis In this study employing sodium bicarbonate rather than sodium lactate to obtain a significant alkalosis the mortality rate following coronary occlusion was the same as the control but the fibrillation rate of 60% was twice the control level Thus alkalosis as obtained with sodium bicarbonate does not reduce the fibrillation rate which follows the demanding stimulus of coronary occlusion

(6) **Hypercalcemia** Mortality 80% fibrillation 60% The infusion of 1 gm calcium chloride elevated the serum calcium from a mean control of 5 mEq /L to 7 7 mEq /L

(7) **Excess Sodium Chloride** Mortality 100% fibrillation 80% Only 500 cc of 0 9 sodium chloride was infused and this hardly represents an excess as demonstrated by the *minimum* rise in sodium and chloride Likewise the other ions measured were not appreciable changed

(8) **Procaine.** Mortality 100% fibrillation 100% Procaine administered intravenously as Novacaine 500 mg was the most lethal agent used in this study The topical administration of procaine was not investigated

(9) **Papaverine** Mortality 60% fibrillation 60% Although papaverine has been reported to raise the threshold for ventricular fibrillation and has been demonstrated to be a marked coronary dilator the fibrillation

rate was twice the control level following the intravenous administration of 30 mg of this drug.

(10) **Quinidine.** Mortality 60%, fibrillation 60%. In some of the older literature, quinidine was believed to be beneficial following coronary thrombosis. In our series, in which we employed 100 mg of the drug intravenously, the fibrillation and mortality rates would suggest that it offers no protection against the development of ventricular fibrillation following coronary occlusion.

(11) **Growth Hormone.** Growth hormone, 100 mg was administered intramuscularly, 12, 4, and 1 hour preceding coronary occlusion. A mortality of 80% and fibrillation of 40% resulted. When 100 mg of the drug was administered 12 and 4 hours preocclusion, a 60% mortality rate and 60% fibrillation rate resulted. Watkins *et al*, in an experiment similar to this report, showed a mortality rate less than 40% and a fibrillation rate less than 20%, following the administration of 200 mg of growth hormone. Their study differed from ours only in the fact that coronary occlusion was produced at the time of initial operation, whereas in this report an interval of 24 hours intervened. In our study, growth hormone failed to decrease the mortality or fibrillation rates over that of the controls.

SUMMARY

1. The effect of selected drugs and various inorganic ions on ventricular fibrillation and mortality following experimental coronary artery occlusion has been investigated.

THE LIMITS OF MYOCARDIAL TOLERANCE TO TOTAL CORONARY OCCLUSION*

WATTS R WEBB AND HECTOR S HOWARD

The resistance of the myocardium to total acute ischemia has been regarded as limited by its high intrinsic metabolic demands Persistent myocardial changes occur very quickly following temporary occlusion of the coronary arteries Persistent electrocardiographic changes were found in dogs by Blumgart and co workers[1] if a coronary was occluded for as little as 15 minutes If occlusion was greater than 25 minutes, an infarct was dem onstrable, and after 45 minutes the results were the same as if the occlusion had been permanent Other workers results have been comparable

Wesolowski and his associates[2] subjected dogs to total body perfusion for 30 and 60 minute periods during which the heart was excluded from the circulation Eight of 19 dogs survived the first day with 4 long term survivors This would indicate that 30 minutes of total occlusion might be beyond the tolerance of the dog s heart in most instances

Clinical experience has shown that the heparinized heart which has been stopped with one of the cardioplegic drugs has a longer total possible period of ischemia

It has usually been accepted that these periods represent the upper limits of cardiac tolerance because of irreversible degeneration of the myocardial tissue itself The work of Crowell and coworkers,[3] however, has demon strated that the accumulating acids of anaerobic metabolism cause in numerable small blood clots to form in the blood vessels within 3 to 5 minutes of circulatory arrest Even though major circulation is re estab lished, these plugged capillaries deprive many areas of oxygen and nutrition These tissues must eventually die though irreversible changes may not have occurred during the period of circulatory arrest Prevention of this *in vivo* coagulation by massive doses of heparin or by activating a fibrinolytic system with streptokinase will considerably prolong the period a tissue may be deprived of normal circulation Thus they have been able to achieve total recovery of dogs — including cerebral function — after 15 minutes of cardiac arrest

The following experiments were designed to study the effects of reversible anoxia on myocardial tissue when the possibility of small capillary throm boses had been obviated by removing all blood from the coronary vas cular bed

METHOD

Series 1. In preliminary experiments hearts were transplanted from small mongrel dogs to the neck vessels of larger dogs in the following manner[4] The brachiocephalic artery of the donor heart was anastomosed to the distal end of the host carotid artery, the left pulmonary artery to the prox imal end of the host jugular vein, and the left auricle to the proximal end of the host carotid artery Circulation was restored after 90 minutes of exteriorization The three controls, with blood remaining in the coronary

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vessels, fibrillated on restoration of coronary flow but none showed return of adequate cardiac tone and none could be resuscitated Four hearts had the blood completely washed out with lactated Ringer's solution Each of these hearts, on restoration of circulation, also fibrillated but developed good tone following calcium chloride injections Each, after defibrillation, was able to maintain the work load of its own coronary inflow and, in addition, the burden of the inflow from the host carotid artery

Series 2. As an evaluation of the ability of the heart to sustain its usual work load, the following experiments were performed

A sternal splitting thoracotomy was performed and the heart and lungs isolated The heart, trachea and lungs were dissected completely free within the mediastinum except for major structures and separated from each other insofar as possible This was found necessary to prevent the return of bronchial and other collateral circulation to the pulmonary veins and thus to the coronaries Aspiration of the left atrium was found inadequate for complete removal of this blood The cavae and ascending aorta were clamped Lactated Ringer's solution was perfused through the right atrium and allowed to empty through a small incision made in the ascending aorta proximal to the clamp After completion of the washout of the heart and lungs, the small incision in the ascending aorta was repaired and after a 90 minute period, circulation was restored to the heart

During the period of cardiac exclusion the dog was maintained by a pump oxygenator, adjusted to maintain a low normal blood pressure and normal temperature

Control experiments were done in exactly the same manner even with the initial perfusion of lactated Ringer's solution but collateral circulation was allowed to return via the bronchial circulation and thus fill the coronaries

OBSERVATIONS

With perfusion the lungs became snow white in color and the hearts translucent Contractions continued unchanged for 2 to 5 minutes, then the hearts gradually slowed and became flaccid Occasionally the ventricles fibrillated but more frequently they stopped completely after 5 to 10 minutes

Many technical difficulties were encountered during the conduction of these experiments both regarding the surgical procedures and the problem of oozing from the large raw surfaces of dissection There were 18 technically successful experiments in which complete bypass was maintained for a 90 minute period

RESULTS

In the 6 control dogs, after the period of occlusion, all hearts improved in color and developed fine fibrillation Only 3 of the hearts were able to return to a normal rhythm as the other 3 did not develop sufficient tone to be resuscitated None was able to maintain the work load of the total circulation once the pump oxygenator had been discontinued

The 12 perfused hearts usually started with fine fibrillation, though one started with a spontaneous coordinated rhythm Tone was improved by calcium chloride and adrenalin, and the hearts were defibrillated with

an electric shock of 180 volts of 0.1 second duration. Eleven were able to maintain a normal blood pressure while one heart with recurrent fibrillation failed under the work load. The last 11 dogs survived from 15 minutes to 14 hours. Electrocardiograms returned to a fairly normal pattern except for evidence of anoxia.[5]

Even though cardiac function could be restored, no long-term survivals were achieved in this group because of the surprising finding that these dogs were unable to establish an adequate breathing pattern but could make only temporary gasping attempts at respiration. To determine if total denervation of the lung might be the underlying difficulty, other dogs were subjected to the initial operative preparation with complete division of all mediastinal connections to the heart, trachea and lungs but without the cardiac bypass. These were unable to resume self-sufficient respirations even though the phrenic nerves were preserved intact. Many further experiments on various phases of denervation of the lung have all supported this finding that the dog with total denervation of the lung is unable to maintain adequate respiration.[6]

DISCUSSION

The time limit of viability of the unoxygenated myocardium is at least in part related to preservation of a patent capillary bed and not alone to the length of acute ischemia. Previous survival experiments have been more a measure of the time required for an irreparably damaging number of capillaries to be thrombosed than a measure of the intrinsic tolerance of the myocardium itself to ischemia.

The above finding that a heart can remain viable and functional for these periods of time appears to remove, at least for experimental work, a previous limiting time factor. In many subsequent experiments with cardiac transplantation utilizing this principle for time periods up to 105 minutes, restoration of the functional capacity of the heart has not been a problem.

Many workers previously have utilized the principle of perfusing the blood from an organ during transplantation. The only similar work on the heart we have been able to find is an accidental observation by Marcus, Wong and Luisada.[4] In their experiments transplanting a heart to the neck of another dog, coronary circulation was maintained by cross-circulation from a third dog. One heart which had been washed in tap water was re-implanted "just for practice" after 45 minutes and, to their amazement, returned to a strong coordinated beat.

Obviously, a full evaluation of the ischemic heart must await a technique which will produce longer survivals A heart that has been subjected to major trauma, whether ischemic, chemical, thermal or surgical, is not necessarily safe from the complications of arrhythmia, failure, or infarction after a few hours.

SUMMARY

1. Perfusion of the coronary vessels to remove all blood and prevent intravascular clotting extends the period of time that the heart may remain viable without coronary circulation.

2. The time limits of viability of the unoxygenated myocardium are

related in large part to the preservation of a patent capillary bed and not solely to the length of acute ischemia

3 Under the conditions of these experiments dog hearts may be without coronary circulation up to 90 minutes and resume the maintenance of a resting cardiac work load

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THE USE OF THE HEART LUNG MACHINE IN SELECTED CASES OF ACUTE MYOCARDIAL INFARCTION*

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Intra arterial transfusion is a method of treatment which has been reported to be effective in myocardial infarction [1 *] This observation suggested to us that removal of blood from the great veins oxygenation of that blood and return of it to the arterial tree might help patients with myocardial infarction with shock or other manifestations of progressive medically unmanageable deterioration

We feared that the full heparinization needed to utilize the extra corporeal circuit would lead to intramyocardial hemorrhage and extension of the infarct Therefore a series of coronary arterial ligations were performed in dogs in the presence of full heparinization A separate report will give the details with the conclusion that heparinization has no effect on the size of the infarct

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Supported by grants from the American Heart Association the New York Heart Association the United States Public Health Service and the Life Insurance Medical Research Fund

Selection of patients was made by the medical staff of Kings County Hospital Every effort was made to restrict selection to those patients with electrocardiographically demonstrated infarcts with blood pressure below 80 systolic These patients failed to respond to intravenous l Norepine phrine Bitartrate (Levophed®) showed loss of consciousness and other signs of deterioration

METHOD

The apparatus used and the method of cannulation of the venous and arterial trees of the patient have been previously described together with successful use for intractable heart failure [3]

Heparin is administered to the patient in the amount of 2 5 mg /kg All blood is freshly drawn into heparin (20 mg /500 ml blood) Heparin protamine titrations[4] every 30 minutes guide dosage to maintain a blood heparin level of 70 to 90 mg /L

RESULTS

This method has been applied to three patients

Patient 1 — KCH #57 9904 (J Z) a 59 year old man was admitted to Kings County Hospital February 27 1957 after one month of precordial pain on exertion and 12 hours of constant severe pain ECG suggested massive posterior wall infarction Shock was present Continuous intravenous Levophed infusion helped temporarily but after 22 hours he was referred for perfusion because of failure to maintain blood pressure with Levophed and progressive loss of consciousness

At a perfusion rate of 700 to 800 ml of blood per minute a blood pressure of 100 mm Hg systolic could be maintained without Levophed After 3 hours hematuria developed and the perfusion was discontinued A good blood pressure could be maintained without the perfusion even though Levophed was required to do so and the patient remained mentally alert It was possible to wean him from Levophed in 12 additional hours The urine became clear in a period of 5 days He made a slow but steady recovery and was discharged 7 weeks after perfusion

Patient 2 — KCH #57 11069 (J G) This 60 year old man was admitted to Kings County Hospital with a 3 year history of substernal pain on exertion and 12 hours of severe pain with dyspnea He arrived in coma and without detectable blood pressure or pulse Levophed infusion was instituted after a diagnosis of infarction had been established A supraventricular tachycardia of 170 per minute was associated with pulmonary edema and deterioration of his condition Sixteen hours after admission he was transferred for perfusion

Perfusion at about 1 L /min was carried to 7 hours despite the development of hematuria because the patient did not tolerate cessation even with Levophed running During the last 3 hours we fully digitalized the patient thus reducing ventricular rate to about 100/min Following this it was possible for him to maintain his blood pressure with Levophed but without perfusion

It required 2½ days to wean the patient from Levophed Digitalization was continued The urine was clearing when he was transferred to the medical service on the sixth day after perfusion and it was profuse in quantity At 3 00 A M 9 days after perfusion he evaded his nurse and roamed the corridors Upon being returned to bed the patient s blood pressure was unobtainable and was not successfully restored Autopsy was denied

Patient 3 — KCH #57 13940 (E H) This 59 year old man came to Kings County Hospital with a history of a stroke one year before and 24 hours of severe substernal pain A very massive infarct was diagnosed involving septum and anterior and posterior walls with A V dissociation and bundle branch block After 28 hours on the medical service he was transferred because of general deterioration and failure to maintain blood pressure with Levophed

After 4 hours of perfusion with 600 to 800 ml /min it was possible to maintain his blood pressure with Levophed He progressed in encouraging fashion for 30 hours

following which his Levophed requirement progressively increased He died 36 hours after perfusion Autopsy showed complete occlusion of the left circumflex and anterior descending arteries and of the septal artery and partial occlusion of the right coronary artery The only myocardium not undergoing the changes of infarction lay in the right ventricular wall

A fourth patient was perfused in error Autopsy revealed an extensive purulent pericarditis Three other patients were considered candidates for perfusion but died before it was carried out Two of these died within 10 minutes after the perfusion team was called and a third died within 30 minutes

COMMENT

These patients all fit into a group in which our medical department has established a survival rate of not over 15% Perfusion may provide a temporary crutch for the remaining active myocardium and provide arterialized blood under reasonable pressure for the vessels to that myocardium and other organs of the body In one case it sustained the patient until full digitalization could be accomplished Until statistically sound data prove such patients can be salvaged by perfusion further speculation here on mechanisms involved is pointless

SUMMARY

Three patients with acute myocardial infarction and in shock, who did not respond to medical management, were partially perfused on the heart lung machine for periods of 3 to 7 hours All patients survived perfusion One patient was discharged home 7 weeks following perfusion A second patient lived for 9 days and a third for 36 hours Additional cases are to be perfused in an attempt to evaluate this technique

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EVALUATION OF INTERNAL MAMMARY ARTERY LIGATION FOR RELIEF OF ANGINA PECTORIS

CHARLES R BLAIR, ROBERT F ROTH, AND HAROLD A ZINTEL

In February 1957 soon after we became aware of the operation of bilateral internal mammary artery ligation for the relief of angina pectoris, studies were begun on dogs in an attempt to contribute information as to the benefit or lack of benefit of this procedure Articles published in Italian Minerva Medica in 1955 and 1956 presented work by M Battezzati, A Tagliaferro, and G DeMarchi[1,2] which demonstrated, by dye injection techniques, anastomoses between the internal mammary arteries and the coronary arteries through the pericardiophrenic arteries They also reported some of their clinical results and concluded that the procedure was beneficial in humans with angina pectoris

Our studies include acute and long term survivor observations on dogs By use of the method later described we measured total coronary flow with the aorta unclamped, with the aorta clamped, and with both the aorta and internal mammary arteries clamped in the third intercostal space By clamping the aorta and at the same time sucking all blood from the left side of the heart, it was postulated that there should be no coronary flow unless there were outside anastomoses to the coronary arteries If, while the aorta is clamped, the internal mammary arteries are also clamped distal to the origin of the pericardiophrenic artery, one might expect an additional coronary flow if the operation described for relief of angina is actually effective Further, if there is such an additional flow, can it be eliminated by then clamping the internal mammary arteries proximal to the origin of the pericardiophrenic artery? Measurements were made under all of the above conditions

Anatomical studies were carried out using an injectable plastic material and radioactive iodinated serum albumin (RISA) We also were able to demonstrate anastomoses connecting coronary and internal mammary vessels but the results are not included in this report

After some experimentation we decided upon the following method to determine coronary flow which could be measured with or without clamping the internal mammary arteries

METHOD

Nembutal anesthetized dogs on an automatic breathing apparatus were connected to the Dewall type bubble oxygenator with Sigma motor pump set to produce 60% of normal flow of each dog, i e 60 cc./kg

A femoral vein and a femoral artery were catheterized Arterial systolic pressures were recorded in all dogs The mean arterial systolic pressures varied between 100 and 110 mm Hg The dogs were heparinized with a dose of 1 mg /kg injected intravenously *before the carotid* artery and vena cava catheters were inserted

The arterial catheter from the pump was placed in the right carotid artery The venous catheters were placed in the superior and inferior vena cavae The azygos vein was securely ligated

After the pump oxygenator was turned on, a previously prepared 30 cc Foley bag catheter was introduced through a stab wound in the right ventricle and pushed up the pulmonary artery. A purse string suture around the catheter prevented bleeding. The balloon was inflated in the post valvular area. The distal portion of the catheter had previously been plugged and ligated and holes were cut in the catheter proximal to the balloon. Thus all blood which returned to the right side of the heart would run off through this catheter. Suction was not used. This gave a measure of coronary circulation and was recorded in cc/min.

A 5 cc Foley bag catheter, unmodified, was placed on constant suction through a stab wound in the left ventricle. This was used to draw off any blood from the left heart that could possibly find its way into the coronary system. A purse string suture was also placed around this catheter.

The aorta was then doubly clamped above the aortic valve to prevent blood from entering the coronary arteries from the aorta, and after a period of 15 seconds the flow from the right ventricular catheter was measured. For each determination blood was collected over a period of one minute.

The aorta was then unclamped and the heart allowed to have its normal coronary circulation. Multiple repeat measurements were carried out both with the mammary arteries clamped and unclamped.

It is of course recognized that the method used does interfere in part with the mammary coronary anastomotic system since pericardial branches are divided where the pericardium is opened and periaortic branches are occluded when the aorta is clamped. This interference would decrease the possible contributions of these anastomoses.

DISCUSSION

The amount and rate of normal coronary circulation could easily be determined with this setup. In the dogs studied the amount was about *100 cc/min* and the coronary *circulation time was approximately 1 seconds*.

While working out the method of study, it was soon found that with the dog on the pump oxygenator bypass and with the aorta unclamped there could be withdrawn from the left ventricular catheter anywhere from 30 to 120 cc of bright red blood per minute. This was considered to be from aortic valve leakage and from return of bronchial circulation. After the aorta was doubly clamped, the left sided catheter return was 5 to 8 cc/min. This might roughly represent the amount of bronchial circulation return to the left side of the heart.

Four successful acute dog experiments were performed. The internal mammary arteries were exposed bilaterally in the third intercostal space so that they could be clamped or unclamped at will.

Spring operated bulldog type clamps were used for this purpose. Multiple determinations were carried out on each dog. Fifteen measurements with the internal mammary arteries unclamped gave a range of *0 to 1 cc/min* volume with an average of 1 cc. Fourteen measurements with the internal mammary arteries clamped gave a range of 0 to 2 cc/min volume with an average of a little less than 1 cc (Table 1).

Thus in acute experiments there was no significant *coronary* flow

regardless of whether the internal mammary arteries were clamped or unclamped

In February, 1957, 6 dogs were prepared for long term survivor studies by bilateral extrapleural internal mammary artery ligation in the third intercostal space Five of these dogs were suitable for study 6 months later

A total of 39 measurements were performed on these dogs (Table 2) All of the dogs showed a consistent coronary circulation that averaged from 8 2 to 10 5 cc /min volume with the aorta clamped The average was 9 6 cc This amount is approximately 10% of normal coronary flow

On the last 2 dogs studied several determinations were carried out with the internal mammary arteries temporarily clamped at their origin This theoretically would eliminate any contribution of mammary coronary anastomoses These measurements were alternated with the previously described measurements It was found that such clamping resulted in a flow of less than 1 cc./min The measurements unclamped would return to their usual average of 9 to 10 cc A total of 8 such determinations were made

Table 1 Results in Dogs, Acute Experiments, With and Without Bilateral Internal Mammary Artery Ligation in the Third Intercostal Space

PUMP OXYGENATOR	Four Dogs 12 to 20 kg 60 cc /kg FLOW MEAN PERFUSION PRESSURE 100 110 mm Hg		
	CORONARY FLOW CC /MIN RANGE	MEAN	AVERAGE
Mammary Arteries Clamped 15 determinations	0 4	1	1 1
Mammary Arteries Unclamped 14 determinations	0 2	0	0 6

Table 2 Results in Dogs Six Months Post Bilateral Internal Mammary Artery Ligation in Third Intercostal Space

PUMP OXYGENATOR	Five Dogs 12.3 to 16 4 kg 60 cc /kg FLOW	Total of 39 measurements PUMP FLOW	MEAN PERFUSION PRESSURE 100 110 MM	
DOG	kg	CC /MIN	CORONARY FLOW CC /MIN AVERAGE	MEAN
1	13.3	800	9.3	9.5
2	16 4	1000	10.5	9.5
3	14.5	870	10	10
4	13.2	800	10	10
5	12.3	740	8.2	9
All dogs	13 9	830	9 6	9.5

Note In acute experiments with internal mammary arteries clamped at their origin the coronary flow averaged less than 1 cc /min

CONCLUSION

It is thus suggestive that bilateral internal mammary artery ligation in *the third intercostal space in dogs does lead* to a significant contribution of arterial blood from the extracardiac mammary circulation to the coronary circulation

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OBSERVATIONS ON CONTROLLED CARDIAC ASYSTOLE IN INTACT DOGS*

ROBERT J SCHRAMEL, ERSKINE ROSS, R D MORTON, AND OSCAR CREECH, JR

Although elective cardiac arrest with potassium ions and acetylcholine[1] *has been accepted as an aid to open intracardiac surgery, it is desirable to* evaluate the effects of this procedure in the absence of a complicating thoracotomy and cardiotomy A method has been developed which permits observation of the mechanisms of arrest and recovery in an intact animal while circulation is maintained by means of a pump oxygenator This report is concerned with the details of the method and experimental results obtained

There were two general objectives to this investigation First, we wished to determine if it was feasible to produce elective cessation of cardiac activity in intact animals under controlled conditions with survival of the animal and without evidence of cardiac injury Second, we have tried to evaluate the action of a variety of cardioplegic substances in the absence of complicating thoracotomy and cardiotomy

METHOD

Adult mongrel dogs weighing in excess of 12 5 kg were anesthetized with intravenous nembutal (15 mg /kg) The right external jugular vein, left common carotid artery and the common femoral arteries and veins were exposed Heparin was administered intravenously in a dose of 1 5 mg /kg *Plastic catheters were introduced into the right external* jugular and right common femoral veins and were advanced so that the tip of the superior catheter was at the level of the third intercostal space while the tip of the inferior catheter was at the level of the fourth intercostal space These

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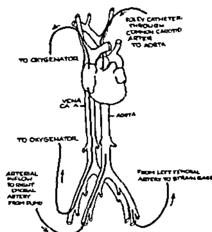


Fig 1 Diagram of the cannulations used to produce elective cardiac arrest in intact animals

catheters were then connected to vinyl tubing of 3⁄16 inch internal diameter which was led through a model T6S Sigma motor pump to a bubble oxygenator The blood in the oxygenator column was exposed to 100% oxygen at a flow rate of about 5 L/min Blood from the oxygenator returned to the right common femoral artery The perfusion was started and adjusted so that the flow of blood was stabilized at the maximum rate possible for the individual animal The left common carotid artery was cannulated with a #8 F Foley catheter This was advanced into the ascending aorta The bag on the catheter was inflated with 3 cc of saline and gentle traction exerted on the catheter until it was felt to lock at the opening of the brachiocephalic artery The bag was then inflated with 6 to 7 cc (total) of saline to occlude the ascending aorta Injections into the coronary circulation were made through the Foley catheter

Arterial pressures and the electrocardiogram were continuously recorded by a multichannel direct writing oscillograph

Potassium citrate was used to produce cardiac arrest in 17 dogs This was given as 25% solution dissolved in whole blood 1 4 A number of chemical agents including 50% glucose 2 molar sodium lactate, 10% calcium chloride and adenosinetriphosphate (ATP) were used to assist the recovery of the K arrested heart Mecholyl or acetylcholine (ACH) was used to produce cardiac arrest in an additional 8 dogs When mecholyl was used in most instances a preliminary injection of 0 5 mg prostigmine was given intravenously shortly before placing the atrial catheters Atropine sulfate was used to assist in resuscitating the heart paralyzed with mecholyl In three animals adenosine[3] was used in attempts to produce cardiac arrest

RESULTS

Cardiac standstill was readily produced in all animals given potassium and there was no tendency to establish any regular electrical activity until aortic occlusion was released Resuscitation of the K arrested heart was difficult Only 6 of 17 animals survived (Table 1) All but 2 of the others developed ventricular fibrillation Chemical defibrillation with more potassium was readily achieved but fibrillation invariably recurred *In dogs 7 8 9 10* glucose solution 50% with insulin was injected into the aorta after aortic occlusion had been released for a few minutes This agent did not prevent fibrillation In dogs 8 and 9 molar sodium lactate (5 cc) was

placed directly in the coronary circulation after fibrillation had become established This did not abolish fibrillation ATP dissolved in Ringer's solution was instilled directly into the coronary circulation of 3 potassium arrested hearts None of these animals fibrillated One given 75 mg survived The 2 which expired were unable to maintain an adequate blood pressure when the pump was turned off Furthermore they exhibited pronounced "potassium effects" despite prolonged perfusion with oxygenated blood

Mecholyl, while producing arrest for a short period, did not result in controllable complete cessation of electrical activity Slow ventricular complexes at rates of 10 to 20/min persisted after an initial 25 to 60 seconds of complete electrical inactivity Resuscitation with this drug is no problem, however, since a normal EKG is readily obtained by the administration of atropine 0 5 to 1 0 mg No instance of ventricular fibrillation was observed with this drug (Table 2) Two animals receiving 5 mg of mecholyl were unable to maintain an adequate blood pressure despite an essentially normal EKG Both expired The other death in

Table 1 Results of Experiments with Potassium Citrate

DOG	WEIGHT	POTASSIUM CITRATE SOL (CC)	DURATION OF ARREST (MIN)	DURATION AORTIC OCCLUSION	DURATION OF PERFUSION	RESULT
1	12 5	5	24	6	60	Survived
2	17 9	6 5	32	5	70	Survived
3	13 0	5	9	6	45	Vent Fibrillation
4	14 0	5	18	11	85	Vent Fibrillation
5	16 9	4	22	5	45	Vent Fibrillation
6	16 0	10	20	5	118	Survived
7	17 0	5	14	5	37	Survived
8	18 5	3 5	12	5	75	Vent Fibrillation
9	17 0	3 5	18	5	75	Vent Fibrillation
10	16 0	3 5	15	5	90	Vent Fibrillation
11	14 0	1 5	6	5	98	Vent Fibrillation
12	16 0	2 8	30	10	55	Survived†
13	14 5	4	9	5	60	Vent Fibrillation
14	14 5	5	22	21	27	Vent Fibrillation
15††	13 0	2	7	5	20	Survived
16††	11 5	4	35	15	55	Expired
17††	12 0	4	23	16	60	Expired

†K + ATP

††K dissolved in normal saline

Table 2 Results of Experiments with Acetylcholine and Mecholyl

DOG	WEIGHT Kg	ACETYCHOLINE (ACH) MECHOLYL (M) Mg	DURATION OF ARREST (min)	DURATION AORTIC OCCLUSION (min)	DURATION OF PERFUSION	RESULT
1	24 0	2M	2	2	20	Survived†
2	13.5	3M	6	6	22	Expired
3	14.5	2M	7.5	7.5	20	Survived
4	14	200 ACH	1	1	20	Survived†
5	17	3M	10	10	22	Survived
6	17	3M	5	5	20	Survived
7	12	5M	2	[illegible]	90	Expired
8	14 0	5M	15	15	45	Expired

†*Received no prostigmine*

this group occurred after an apparently successful experiment as the dog was being transferred to the animal house

Adenosine[3] (200 mg) was infused into the coronary circulation of 3 animals Only a transient bradycardia was observed All the animals survived

CONCLUSIONS

Cardiac arrest can be produced in intact animals by the method described with survival of the animals This method may be of value in the evalua tion of cardiac drugs other than those producing arrest Neither potassium ions nor mecholyl are completely satisfactory substances for producing cardiac arrest

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COMPARISON OF THE RESPONSE TO POTASSIUM CARDIAC ARREST OF HEARTS UNDER HYPOTHERMIC AND NORMOTHERMIC CONDITIONS*

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The purpose of this series of experiments was to evaluate the effect of hypothermia upon the production of cardiac arrest with potassium citrate and upon the restoration of the cardiac action following this arrest.

Elective cardiac arrest was induced by the method of Melrose and associates.[1] Their method has been confirmed at normothermic levels both in dogs and humans by Kolff, Effler, *et al.*[2, 3] Sealy and associates[4] have recently reported a clinical case of elective cardioplegia during hypothermia. They used a solution of potassium citrate, magnesium sulfate, and prostigmine to produce the cardiac arrest. There has, however, been no report in the literature as yet comparing the relative safety of this method of arrest at hypothermic versus normothermic temperatures.

METHOD

Two groups of mongrel dogs were used in this experiment. The dogs varied in weight from 25 to 50 pounds. The dogs were anesthetized with the intraperitoneal injection of 33 mg./lb. of nembutal. The trachea was intubated and respirations were maintained with a mechanical respirator.

The first group of dogs was studied at hypothermic levels. Each was placed in a cold water bath and within an hour the body temperature as measured per rectum was dropped to approximately 30°C. At this temperature they were removed from the water bath and further drop of the temperature was prevented with a heating pad. The second group was studied at normal temperature. This was maintained during the entire procedure with the heating pad. The temperature of each dog was constantly monitored with a rectal thermocouple. All animals were subjected to the same subsequent procedure.

A bilateral trans-sternal thoracotomy was done entering the chest through the third or fourth intercostal space. The pericardium was widely opened, and the left subclavian artery, and the superior and inferior vena cava were isolated. The aorta was dissected from the pulmonary outflow tract. Tapes were placed around the aorta and around the pulmonary artery. The two cavae and the left subclavian artery were cannulated with plastic catheters. The dogs were then perfused using a bubble type oxygenator and Sigma motor pump at a flow rate of 25 cc./kg./min.

The aorta was occluded 2.5 to 3.0 cm. from its origin with a clamp. Two cubic centimeters of 25% potassium citrate was mixed with 18 cc. of blood and the resulting 2.5% potassium citrate solution was quickly injected into the root of the aorta proximal to the occluding clamp. The injection was continued until the heart went into asystole. The pulmonary artery was immediately occluded and a right ventriculotomy was done.

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The heart was closely observed for any sign of ventricular activity At the end of 16 minutes of asystole the aortic clamp was removed and the ventriculotomy wound was closed The last suture was taken after the ventricle had begun to contract so that all the potassium bearing blood coming from the coronary system was washed out of the heart

The time of the first ventricular contraction and the time of restoration of the heart to a normal sinus rhythm were both noted As soon as the heart was back to a sinus rhythm the perfusion was stopped, and the cannulas were removed from the subclavian artery and the cavae The thoracotomy wound was closed and the dogs were sacrificed after a one hour period of observation

RESULTS

The time required for the arrest to occur was measured from the moment of injection of the citrate into the aorta until the heart went into asystole The time required for restoration of cardiac function was measured from the moment of removal of the aortic clamp until the heart returned to a sinus rhythm with a rate of more than 60 beats per minute

The results are shown in the following table

Table 1

	NUMBER OF ANIMALS	AV TEMP °C	AV DOSE K CITRATE REQUIRED FOR ARREST	AV TIME FOR ARREST TO OCCUR	AV TIME FOR RESTORATION TO SINUS RHYTHM	INCIDENCE OF VENTRICULAR FIBRILLATION
Normothermia	8	36 2°	240 mg	25 sec	3 6 min	2 dogs
Hypothermia	10	29 8°	605 mg †	91 sec ††	25 7 min †††	1 dog

†175 to 2500 mgs
††8 to 420 sec
†††4 to 71 min

The average of the results in each series of experiments is recorded in the table In the normothermic group there is very little deviation of any of the observations from the average The dosage of potassium citrate varied from 175 to 500 mg The time required for arrest to occur in this normothermic group ranged from 6 to 90 seconds, and the time for restoration to a sinus rhythm varied from 30 seconds to 9 minutes However, in the hypothermic group there are wider deviations from the average The dosage of the potassium citrate varied from 175 to 2500 mg and similarly the time for asystole to occur ranged from 8 seconds to 7 minutes The time for restoration of the heart to a sinus rhythm varied from 4 to 71 minutes in this group Ventricular fibrillation occurred in 2 of the normothermic dogs just after the aortic clamp was removed One was immediately rearrested with potassium citrate and the other was given electric shock Both returned to a sinus rhythm within six minutes Ventricular fibrillation occurred in one of the hypothermic dogs 13 minutes after the aortic occluding clamp had been removed The heart was again arrested with

potassium returning to sinus rhythm 24 minutes later under appropriate treatment

DISCUSSION

The qualitative response of the heart to the intracoronary injection of potassium citrate and its subsequent removal by perfusion was not altered by hypothermia in this experiment However a larger dosage of potassium citrate was usually required to induce asystole This larger dose requirement may be only apparent This is because with the slow response of the heart to potassium larger doses may be injected since the injection is continued until the heart stops The resistance of the heart to restoration to a sinus rhythm may be due to prolongation by the low temperature of the chemical metabolic reaction involving potassium or due to the fact that more potassium citrate was required in the first place to produce asystole

The incidence of ventricular fibrillation in the two groups is not significantly different and recovery was accomplished in each instance

The results of this experiment offer some encouragement for the clinical use of potassium cardiac arrest during open cardiotomy with hypothermia

SUMMARY

1 *A heart at normothermia requires on the average a smaller dosage* of potassium citrate and a shorter time for elective cardiac arrest to occur than does the hypothermic heart

2 The normothermic heart recovers from cardiac arrest more quickly with earlier initial ventricular contractions and with a faster return to a sinus rhythm than does the hypothermic heart

3 The comparative incidence of ventricular fibrillation is not statistically significant being 2 in a series of 8 normothermic dogs and 1 in a series of 10 hypothermic dogs

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LOCALIZED CARDIAC HYPOTHERMIA AS AN ADJUNCT TO ELECTIVE CARDIAC ARREST*

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Elective cardiac arrest with potassium citrate has become an accepted adjunct to open cardiac surgery However, since there has been criticism of this technique, it is the purpose of the present report to re evaluate potassium citrate arrest, as well as the use of localized cardiac hypothermia with and without potassium induced cardiac arrest during periods of coronary artery inflow stasis

METHOD

Experiments were performed on mongrel dogs anesthetized with pento barbital, 30 mg /kg The right thoracic cavity was entered through the fourth interspace and artificial respiration maintained with compressed air The animals were heparinized with 1 65 mg of heparin per kg following which catheters were placed for cardiac bypass During the period of cardiac bypass, circulation was maintained by a rotating disc reservoir oxygenator coupled with a Sigma motor pump[1] Mean arterial pressure was registered with a mercury manometer, and superior vena cava pressure with a saline manometer Cardiac arrest was produced by cross clamping the ascending aorta and main pulmonary artery and injecting potassium citrate utilizing 2 cc of a 25% solution in 18 cc of blood, into the ascending aorta proximal to the clamp Cooling of the myocardium was accomplished by infusing 100 to 200 cc of arterial blood at 0°C at a pressure of 100 to 200 mm of mercury through a 15 gauge needle into the occluded segment of the proximal aorta During the period of aortic occlusion the proximal segment of aorta was vented in order to prevent perfusion of the coronary arteries by blood reaching the left heart via the bronchial vessels Myo cardial temperature was recorded by a Leeds and Northrup Speedomax from a thermocouple placed in the wall of the right ventricle

Results were evaluated on the basis of 1) the incidence of ventricular fibrillation or other abnormal rhythm developing after coronary blood flow was re established, 2) duration of pump support time before the heart could take over its function following re establishment of coronary circulation, 3) the ability of the heart to maintain adequate circulation as indicated by mean arterial and venous pressures following *cessation of* the pump support, 4) changes in the electrocardiogram and the duration of such changes

RESULTS

Controls In 12 dogs, the coronary blood supply was occluded for periods of 20, 30 and 40 minutes Ventricular fibrillation occurred in each instance within 10 minutes after clamping the aorta (Table 1) Following re estab lishment of coronary flow, defibrillation was accomplished readily by electric shock The circulation had to be supported by the pump for

*From the Department of Surgical Research in the Division of Surgery St Lukes Hospital Cleveland Ohio Supported in part by the Elisabeth Severance Prentiss Founda tion The Cleveland Area Heart Society and the Life Insurance Medical Research Fund

Table I

DURATION AORTIC OCCLUSION MINUTES	NUMBER OF EXPERIMENTS	VENTRICULAR FIBRILLATION	PUMP SUPPORT†††† TIME MINUTES	FINAL MEAN ARTERIAL PRESSURE MM Hg	FINAL VENOUS PRESSURE MM SALINE †††	MYOCARDIAL TEMPERATURE INITIAL 0°C	MYOCARDIAL TEMPERATURE FINAL	ECG —
Controls								
20	4	4	10 (6 5 17)	79 (70 94)	35 (10-60)	37	37	Normal within 28 minutes in 1 dog
30	1	1	7.5	80	45	37	37	
40	7	7	16 5 (8 25)	67 (40 88)	42 (25 60)	37	37	Normal in 30 to 60 minutes in 6 dogs
Potassium Arrest								
10	10	7†	17 (5 30)	71 (30 110)	57 (0 180)	37	37	
20	1	1††				37	37	
40	3	3	40 (9 60)	27 (16 60)	87 (20 140)	37	37	Abnormal at 44 and 80 minutes in 2 dogs
Cardiac Hypothermia								
13	2	2	95 (9 10)	86 (90 92)	7.5 (0 15)	19 5	28	
21	3	3	9 (7 10)	93 (90 100)	10 (5 15)	20	28	
40	1	1	12	125	10	22	29 5	
Potassium Arrest Followed by Cardiac Hypothermia								
20	3	0	10 (5 14)	105 (95 120)	40 (20 50)	18	28	
40	7	2	5 (3 8)	90 (80 110)	11 (0 30)	18	29	Normal in 2 to 6 minutes in 7 dogs

†In two experiments heart could not be defibrillated
††Could not be defibrillated
†††Values expressed as increase above pre occlusion level
††††Time from release of aorta

8 to 25 minutes (average 16 5) after the defibrillation to prevent a sharp fall in arterial pressure and a rise in central venous pressure associated with marked cardiac distension In those experiments in which the aorta was occluded for 40 minutes, the average mean arterial pressure 30 to 60 minutes after cessation of pump support was 67 mm Hg The average superior vena cava pressure was 12 mm of saline higher than the control values obtained before placing the dogs on the bypass Electrocardiograms on 6 of 7 dogs in the 40 minute occlusion group did not return to pre operative control patterns for from 30 to 60 minutes after the heart had been defibrillated

Potassium arrest and cardiac hypothermia. In 10 dogs, the heart was arrested by the injection of potassium citrate (Table 1) The potassium was injected until there was no cardiac activity as judged by direct observa tion and/or electrocardiograms Upon release of the aorta, 11 of the dogs immediately developed ventricular fibrillation Two of these animals could not be defibrillated by electric shock or by the use of a second injection of potassium citrate followed by electric shock and calcium chloride The aorta was occluded for only 10 mintues in the 3 dogs which did not show ventricular fibrillation Pump support time for those dogs that could be defibrillated was longer than that required for the control animals Arterial pressure was lower and central venous pressure higher than in the control dogs at comparable periods of time following restoration of normal rhythm Electrocardiograms were obtained in 2 dogs after occlusion of the aorta for 40 minutes In one animal, the electrocardiogram did not become normal until 80 minutes after defibrillation, and in the other, an abnormal record was still present 44 minutes after defibrillation when the experiment was terminated

Cardiac hypothermia. The myocardium was cooled to about 20°C by the infusion of cold blood into the proximal segment of the occluded ascending aorta in 5 dogs The coronary circulation was interrupted for 13 minutes in 2 dogs, 20 minutes in 3 dogs, and 40 minutes in 1 Ventricular fibrillation occurred in all dogs in this series during the period of aortic occlusion Defibrillation was easily accomplished in all animals with electric shock following re-establishment of coronary circulation Pump support time was not significantly different from the control dogs However, arterial pressures were higher and the central venous pressures lower than in the control group 30 to 60 minutes after the hearts were defibrillated The electrocardiograms of the dog with aortic occlusion for 40 minutes returned to normal 26 minutes after defibrillation

Potassium arrest and cardiac hypothermia. In 10 dogs, the heart was arrested by the injection of potassium citrate into the coronary arteries followed by the intracoronary infusion of blood cooled to 0°C The heart was arrested for 20 minutes in 3 dogs, and for 40 minutes in 7 The temperature of the myocardium was reduced to an average of 18°C, and during the period of cardiac arrest it rose to an average of 29°C None of the dogs in the 20 minute group developed fibrillation on re establish ment of coronary flow, and only 2 of the 7 dogs in the 40 minute group developed fibrillation (Table 1) In these 2 dogs the ventricular fibrillation was abolished by a single pair of shocks In the remainder of the dogs a

regular sinus rhythm supervened within a few seconds of aortic release Post occlusion pump support time in the 40 minute group was significantly less than in the control dogs Arterial pressure was higher and central venous pressure lower than in the control group after the animals had been off the pump for from 30 to 60 minutes The animals with the aorta occluded for 40 minutes showed no electrical activity throughout the period of aortic occlusion except for the appearance of P waves in 1 dog Immediately after release of the aorta, electrocardiograms showed QRS widening ST segment displacement, and T wave inversion, but within 2 to 6 minutes after aortic release, or defibrillation, the electrocardiogram was normal

When the injection of potassium was made after the infusion of cold blood, 4 of 7 dogs with the aorta occluded for 20 minutes showed ventricular fibrillation upon release of the aorta, and in 1 of the animals normal rhythm could not be restored Arterial and venous pressures were similar to those observed in the control group If potassium arrest was followed by the infusion of warm blood, the results were similar to those found in the control group

DISCUSSION

Our results in the control animals are in harmony with those of Wesolowski *et al*[7] who were able to resuscitate hearts made completely ischemic for periods of 30 to 60 minutes

The acute cardiac recovery, as judged by reversal of fibrillation pump support time, arterial and venous pressures, and electrocardiographic changes, was poorer in dogs subjected to potassium arrest than in the control group This would indicate that not only is potassium arrest not a benign procedure, but that it is less well tolerated than interruption of coronary flow in the beating, but nonworking heart These results are at variance with those of Kolff *et al*[3] who observed ventricular fibrillation *in only 1 of 10 dogs subjected to potassium citrate arrest*

Since the rationale of potassium citrate arrest during periods of coronary artery inflow stasis is to reduce myocardial oxygen consumption, it was felt that localized cardiac hypothermia might acomplish the same results without the added risk or inconvenience of generalized hypothermia Ventricular fibrillation occurred in all those animals subjected to cardiac hypothermia alone However, the hearts were defibrillated easily, and the post defibrillation recoveries were better than in the normothermic control group This indicates that some protection was provided by cooling the myocardium By producing cardiac arrest first with potassium citrate and then cooling the myocardium, the high incidence of fibrillation found in *cardiac hypothermia alone was eliminated and cardiac recovery following* re establishment of coronary flow was better The fact that the incidence of fibrillation was greater and the acute recovery less complete when the heart was cooled prior to the administration of potassium indicates that the order in which cold blood and potassium are administered is in part responsible for the favorable results When the former sequence of giving the potassium first is followed, cardiac arrest is obtained with *the potassium* citrate, and the cold blood maintains the arrest while at the same time reducing the potassium ion concentration in the myocardium When the *sequence is reversed and the potassium given* after the cold blood, the

potassium ion concentration in the myocardium remains high and cardiac recovery is slowed the speed of recovery being similar to that obtained when potassium citrate alone is used The beneficial effect of coronary perfusion of cold blood following potassium arrest is not due solely to washing out excess potassium When 100 to 200 cc of blood at 37°C was infused into the coronary arteries following potassium arrest the arrest was not maintained and ventricular fibrillation ensued Since both Swan[4] and Berne[5] have observed a positive potassium balance in the hypothermic heart it is possible that the cooled hearts retain more potassium under the conditions of this experiment On the other hand hearts may remain quiescent at lower concentrations of intracellular potassium

SUMMARY

Elective cardiac arrest produced by coronary artery perfusion with potassium citrate was studied in dogs with normothermic hearts and in those in which the heart was cooled to about 20°C by the infusion of cold blood into the coronary circulation Potassium citrate arrest produced a high incidence of ventricular fibrillation in the recovery period with delayed recovery of normal cardiac action Infusion of cold blood into the coronary arteries following potassium arrest prevented this high incidence of ventricular fibrillation and acute cardiac recoveries as judged by the time of pump support after aortic release post arrest arterial and venous pressures and the electrocardiograms were good Coronary infusion with cold blood alone coronary infusion with cold blood prior to potassium arrest or potassium arrest followed by coronary perfusion with warm blood showed a high incidence of ventricular fibrillation and/or poor post arrest recoveries

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THE TREATMENT OF COMPLETE HEART BLOCK BY THE COMBINED USE OF A MYOCARDIAL ELECTRODE AND AN ARTIFICIAL PACEMAKER*

WILLIAM L WEIRICH, VINCENT L GOTT, AND C WALTON LILLEHEI

The introduction of the pump oxygenator in the field of cardiac surgery has enabled the surgeon to correct congenital anomalies which previously were inoperable. Excellent progress has been made in the correction of even complex defects of the atrial and ventricular septa However, one of the serious complications which have prevented the full realization of optimal results from these curative procedures has been the occurrence of complete atrioventricular block

Isuprel (Winthrop Stearns) has been very helpful in the treatment of this complication [1] However, the response to this drug has been variable, so that the mortality of complete block, while significantly reduced from nearly 100% through the use of Isuprel, still remained high The use of external stimulation of the heart in block through skin electrodes was frequently tried but had to be discarded because of many problems, consisting of pain, burns, and inability to keep up continuous stimulation for the prolonged intervals often necessary in these patients until sinus rhythm is restored For these reasons a more reliable method of electrical stimulation of the heart was sought for and developed

METHOD

Fifty adult mongrel dogs weighing 8 to 26 kg were anesthetized with sodium pentothal Electrocardiographic tracings (Lead II) were recorded prior to and following the production of heart block and during stimulation at various rates In the last 13 successful preparations cardiac outputs and systemic arterial pressures were measured

A #50 polyethylene catheter was introduced into the aorta through the left femoral artery and connected to a *Statham Pressure Transducer* The arterial pressures were recorded on a Sanborn polyviso Cardiac outputs were determined by Hamilton's method of dye dilution [2]

A right lateral thoracotomy was carried out through the bed of the fifth rib Umbilical tapes were placed about the cavae and the azygos vein was ligated with fine silk The pericardium was opened parallel and immediately anterior to the right phrenic nerve Under inflow stasis the right atrium was opened and a single, 3 0 silk suture was inserted across and at right angles to the annulus of the tricuspid valve, 1 0 to 1 5 cm anterior to the coronary sinus, so that the conduction tissue would be incorporated in the suture After the suture was tied the occluding tape on the superior vena cava was loosened and the atrium was allowed to fill with blood The edges of the incision were approximated with a non crushing clamp and the atriotomy was closed with a running 4 0 silk suture

*From the Department of Surgery and the Variety Club Heart Hospital University of Minnesota Medical School Minneapolis Supported by Research Grants from Graduate School University of Minnesota American Heart Association Minnesota Heart Association Life Insurance Medical Research Fund and National Heart Institute Grant #830

In almost all instances complete heart block was produced with one suture Occasionally two sutures were necessary An insulated, silver plated, braided copper wire,† 0 009 inches in diameter, was placed sub epicardially in the wall of the right ventricle This placement has been facilitated by swedging the wire onto a surgical needle The uninsulated segment was fixed to the ventricle with a 4 0 silk suture at the point where it entered and emerged The long segment of the wire, insulated with polyethylene tubing was brought out through the chest wall and fixed to the skin with a fine silk suture The chest was closed in layers and a single rubber thoracotomy tube was used to evacuate the hemithorax

Systemic arterial pressures were repeated one hour after the operation The blood lost by the animal during the procedure and during measure ments of cardiac ouput was replaced with transfusions of whole blood

The pacemaker†† used was a Grass, Model S-4A, Physiologic Stimulator An impulse lasting 2 milliseconds was employed in all the studies The negative electrode was connected to the myocardial wire and the positive electrode was attached to the skin near the apex beat Control of the heart beat was obtained easily with voltages of 0 8 to 9 0 (average, 2 25 volts)

The myocardial wire was removed by traction from some of the animals after the physiologic determinations were completed In the remaining animals the wire was left in place for periods up to 3 weeks No complica tions were observed when the wire was removed The animals were then sacrificed and complete necropsies were performed Minimal tissue reaction was observed about the site where the wire had been inserted in the ventricular wall

Bipolar stimulation by means of two myocardial electrodes was investi gated and found to be equally effective

RESULTS

Systemic arterial pressures. Mean aortic pressures decreased to an average of 70% of pre block values During electrical stimulation of the heart at a frequency of 90/min with low voltages the mean aortic pressures returned to pre block values When the heart was stimulated at rates of 120 to 160/min the mean aortic pressures increased to a maximum of 127% of the control pressures

Cardiac outputs Production of complete heart block reduced the cardiac output to 55% of pre block values During stimulation at rates of 90 to 160/min the cardiac outputs increased from 72 to 167% of the control measurements

Length of stimulation Almost all of the studies were completed in 2 to 2½ hours One animal s heart was controlled by the pacemaker for 24 hours It was not necessary to increase the voltage output of the pace maker during this interval

Site of stimulation There was no difference in the effectiveness of the pacemaker stimulation in animals in which the following sites of implanta tion of the myocardial electrode were studied left ventricle wall, right ventricle wall, and the ventricular septum

†Lenz Electric Manufacturing Co Chicago Illinois

††Most standard pacemakers can be converted without difficulty to this use by an electrician

Clinical experience Since the first clinical use on January 30 1957 this method has been employed in 18 patients with complete atrioventricular block The voltage output of the pacemaker that was required was between 1 5 and 4 5 volts One patient s heart was stimulated for 21 consecutive days before pacemaker stimulation could be safely discontinued No detectible sensations contractions of the skeletal muscle burns infections or other complications from removal of the wire were observed in this group of patients

In this group of 18 patients only one death occurred as a consequence of acute surgically induced complete atrioventricular block This patient a 19 month old infant who underwent repair of a ventricular septal defect was the seventh case in this series of 18 patients Up until and including that case the indifferent electrode had been taped securely to the skin In this infant cardiac arrest occurred when this cutaneous electrode became dislodged while the child was being suctioned The heart was restarted by pressing the electrode onto the skin as soon as the source of the difficulty was appreciated However irreversible neurologic damage had occurred during the interval and death resulted 6 hours later from this complication Henceforth the indifferent electrode has been implanted subcutaneously over the apex of the left ventricle and this complication has not recurred Most of these patients have received Isuprel therapy (linguets administered rectally) in addition to the pacemaker stimulation to maintain a desired positive ionotropic effect upon the myocardium and as a precaution in the event of a power failure

Prior to the use of the pacemaker stimulation with a myocardial electrode our best accomplishments in the treatment of heart block (utilizing Isuprel) had resulted in a reduction of mortality due to heart block to 50% during the postoperative interval Death in practically all cases was due to an inadequate cardiac output The use of the myocardial electrode has brought about a remarkable reduction in this high mortality and morbidity from this complication and has been the single most important factor in reducing the overall mortality of intracardiac surgery to its present low levels

At this writing 40 consecutive patients have had intracardiac reparative surgical procedures utilizing the pump oxygenator with only one death In this series 7 patients had a complete atrioventricular dissociation occurring at the time of operation and all were managed by the myocardial electrode All 7 of these patients reverted to a normal sinus rhythm before discharge from the hospital The techniques for asystole have proved valuable but have substantially increased the incidence of heart block [5] Thus this method for effective control of heart block has become all the more indispensable

A featherweight transistorized pacemaker activated by a dry cell and taped or attached to the patient s trunk has been developed to facilitate transportation of these patients about the hospital and also to allow them more mobility in their convalescent interval in those cases where early return to sinus rhythm has not occurred

SUMMARY

1 In the presence of complete heart block the heart rate was controlled

effectively with the combined use of a myocardial electrode and an artificial pacemaker

2 The mean aortic pressures and cardiac outputs of 13 dogs were restored to preblock levels by this method Only very low voltages were necessary to stimulate the myocardium effectively over prolonged time intervals by this method

3 No complications from insertion and removal of the wire from the myocardium were observed

4 Eighteen patients with surgically induced heart block have been treated by this method One death from acute complete heart block occurred in this group due to a failure in the electrical circuit since corrected by subcutaneous implantation of the indifferent electrode in all cases

5 This remarkable reduction in the mortality associated with complete heart block has been the single most important factor in reducing the overall risk of open heart surgery to low levels

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TOTAL REPLACEMENT OF THE MITRAL VALVE*

ELIOT H BERG STANLEY E GOODMAN JACKSON H STUCKEY AND MELVIN E NEWMAN

For the last 2 years this laboratory has been concerned with the problem of a replacement for the mitral valve The aim has been to create an artificial valve which when placed in the mitral annulus will carry out all of the functions of a normal valve without dependence upon the nature of the pathology of the diseased valve The criteria used in determining a satisfactory prosthesis have been (1) it must stop mitral insufficiency (2) there must be sufficient blood flow without increased left atrial pres sure during diastole to meet the demands of extreme exertion as well as the demands at rest (3) the valve must be durable capable of tolerating approximately 44 million closures annually (4) it must be easily inserted

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(5) the fixation must be permanent, insuring stability between the mitral annulus and the edges of the prosthesis; (6) it should not cause the production of thrombi or emboli; (7) the presence or action of the valve should produce no disturbance of the normal blood elements.

Earlier experience in our laboratory,[1] showed that the plastic ball valve type of prosthesis was not satisfactory in the mitral region because of difficulty in obtaining permanent fixation, and because of the production of thrombi and emboli. The present study is concerned with the application of the flap type of valve, using plastics and metals and employing various types of flap attachment.

METHOD

The insertion of the valve was the same in all cases and similar to that reported by Kernan.[1] Essentially, mongrel dogs weighing 18 to 22 kg. were anesthetized with intravenous pentothal. Respirations were maintained by use of the Jefferson respirator. The chest was entered through the bed of the 5th left rib. Perfusion was accomplished by withdrawal of venous blood from the right atrium and returning oxygenated blood to the left subclavian artery. The method of heparinization, and the pump-oxygenator system employed were those described by Dennis,[2] Newman,[3] and Stuckey.[4] Total bypass of the circulation was obtained by electrically fibrillating the heart.

The mitral valve was approached through an incision in the left atrium which was carried from the appendage to the orifice of the inferior pulmonary vein. The normal mitral valve cusps were excised. Beginning and ending at the lateral aspect of the mitral annulus, a purse string suture of #2 black silk was placed in the annulus. In placing the suture, care was taken not to compromise the coronary artery laterally and anteriorly, the coronary sinus posteriorly, or the aortic valve medially. To prevent the last, a small margin of the mitral cusp was left on the aortic side. The groove cut in the periphery of the prosthesis was placed within the purse string suture. The suture was tied, holding the valve in the annulus. The hinged side of the flap was directed toward the aortic valve.

Once the valve was in place, it was opened and the aortic valve was distorted to allow blood to flush out the air from the left ventricle. The atrial incision was closed using 5-0 continuous atraumatic black silk. The

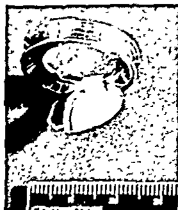


Fig. 1. Mitral Valve Prosthesis

heart was defibrillated The thoracotomy closure, chest drainage, and post operative care were not unusual

The valve form was similar in all cases (Fig 1) It was 1 0 inch in diameter, 250 inch deep, lumen 700 inch, suture groove 080 inch The flap was 750 inch in diameter The thickness of the flap varied from 002 to 017 inch The base was constructed of teflon, nylon, or stainless steel Similarly, the flap was made of either Ivalon,† Ivalon reinforced with spring wire, nylon covered spring wire, nylon covered flat spring metal, or bare flat spring metal All of the above were rigidly fixed to the base, using the spring action of the material to aid in valve closure The later experiments employed a 017 inch stainless steel flap To one edge of this was soldered a 0 4 inch length of 0 050 O D steel tubing The flap was then hinged to the base by a steel wire passing through the walls of the base and the steel tube on the flap A small stop soldered onto the flap prevented opening beyond 70 degrees The ventricular contraction closed the valve satisfactorily

RESULTS

Forty six valves were inserted No animal survived longer than 30 days In the first 5 experiments the base was made of teflon, the flap of pressed Ivalon, either alone or reinforced with spring wire Three died within 24 hours one from hemorrhage, one from myocardial infarction following inclusion of the circumflex coronary artery in the purse string suture, and in one the flap fractured at the fixed edge Two survived 10 days One death was due to occlusion of the valve by clot, the other was due to collapse of the plastic flap In both of these there was extensive clot formation on both the base and the flap

In the next 5 experiments the flap was made of thin watch spring metal or stainless steel 002 inch In one case this was covered with nylon mesh Only one lived for 3 days The flap fractured at the fixed point In those that lived more than one day, there was clot on the base (teflon) but not on the flap (stainless steel)

The next 5 experiments employed a nylon base with a thin spring metal flap Again only one lived 3 days Death was due to fracture of the flap Clot was present on the base but not on the flap

The next 9 experiments employed a stainless steel base, with several modifications of the method of attachment of the spring metal flap None lived more than 24 hours When death was not due to surgical error (3 cases) it was due to fracture of the flap at the fixed point For the short time that the metal base was in the blood stream, no clot was formed

At this point the flap was constructed of heavier steel (017 in) and placed in the stainless steel base on the free swinging hinge described above Twenty two experiments were carried out In the last 3 a wire mesh was soldered into the suture groove, to improve fixation The length of survival, cause of death, and evidence of emboli are shown in Table 1 In two of the survivals of less than 10 days (14 cases) death was due to the valve The first was a poorly constructed valve preventing free action of the flap In the other instance the valve slipped out of the purse string

†Polyvinyl sponge obtained from Clay Adams Co

Table 1 Length of Survival, Cause of Death and Evidence of Emboli (as Shown by Hematuria or Renal Infarcts) in the Last 22 Cases

Mitral Valve (Stainless Steel Flap and Base)

DAYS OF SURVIVAL		0	1 2	3 9	10 19	20 30
NUMBER OF EXPERIMENTS		8	1	5	5	3
CAUSE OF DEATH: Surgical Error or Medical		7		5		
Poorly Constructed Valve		1				
Fixation Failure			1		2	1
Fibrosis at Hinge, Preventing Flap Function					3	2
Hematuria Prior to Death		0	0	0	0	0
Renal Infarcts	Yes			2	1	2 (One Massive)
at Autopsy	No		0	3	4	1

suture In those surviving 10 to 30 days (8 cases) the deaths were due to failure of the purse string suture in 3, and fibrosis at the hinge, preventing flap action, in 5 In this group there was thrombus only at the hinge The remainder of the circumference of the base, exposed to the flowing blood stream, was free of clot Clot invariably formed where the wire mesh was soldered into the suture groove

DISCUSSION

The first 24 experiments performed suggested that stainless steel, both as the base and the flap, has much less tendency to produce clot than the plastics To evaluate this, a free floating flap was sutured in the lumen of the abdominal aorta On removal after one month, there was no clot formation on the metal or on the adjacent vessel wall

The first group of experiments also made it evident that a prosthesis can be closed in the same manner as the normal valve is closed, that is by the ventricular contraction An added spring mechanism merely jeopardizes the flap

The form of the valve used in the last 22 experiments is such that life time construction offers no mechanical difficulty The two problems at present are permanent fixation and clot formation Various methods of milling of the suture groove, or installation of a mesh in the base of the groove may offer a more secure and stable fixation

Clot formation may be due to either the form of the valve or the rather crude construction To eliminate the latter, the valve in Figure 1 was made, constructed entirely of stainless steel and with highly polished surfaces † The results of the application of this valve have not been ascertained as yet

†Made by the Bulova Watch Co

SUMMARY

A flap valve is described, which may be suitable for replacing the mitral valve The results of its application in 46 experiments are presented The longest survival time was 30 days Mechanically the valve is satisfactory The problems of clot formation and permanent fixation are considered

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TRANSARTERIAL PULMONARY VALVULOTOMY IN THE FUNCTIONING HEART A DIGITAL AND INSTRUMENTAL APPROACH THROUGH A DIVERTICULUM*

WILLIAM W L GLENN, HERBERT S HARNED, JR, AND ALLAN V N. GOODYER

Valvulotomy for the relief of congenital pulmonic stenosis was first attempted in 1913 by Tuffier who introduced a knife through the wall of the right ventricle Brock[1] was the first to accomplish pulmonary valvulotomy by the transventricular route His technique has been modified by Potts[2] and others Sondergaard[3] opened the stenosed pulmonary valve *from above* through the pulmonary artery He worked through a rubber extension attached by a special ring clamp to the wall of the pulmonary artery A modification of this technique was reported by Glenn,[4] *et al* Pulmonary valvulotomy under direct vision was reported by Dodrill[5] using an extracorporeal circulation and by Swan[6] using vena caval occlusion and hypothermia

The purpose of this communication is to report our experience with transarterial pulmonary valvulotomy performed by a digital and instrumental technique through a diverticulum sutured to the pulmonary artery

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METHOD

Thirty patients were operated upon with the diagnosis of pulmonary valvular stenosis between January, 1952 and July 1956 A transventricular (TV) approach to the pulmonary valve was used in 17 patients and a trans arterial (TA) approach in 13 patients The average age of the TV group was 13 3 years and of the TA group was 20 5 years Eighteen of the total number of patients were males and 13 were females The same operator performed or supervised all of the operations

The technique. Under general endotracheal anesthesia with the patient lying on the right side in a full lateral position, the chest is entered through a long incision removing most of the 4th rib The pericardial sac is opened anterior to the left phrenic nerve exposing the pulmonary artery and the outflow tract of the right ventricle The presence of pulmonary valvular stenosis is confirmed by palpation through the wall of the pulmonary artery of the thickened and often cone shaped valve cusps and by a strong systolic jet as the blood is forced through the constricted valve orifice

A 1 to 2 cm wide section of the anterior wall of the pulmonary artery approximately 5 cm in length is grasped in a clamp of the Satinski Glover design The toe of the clamp is applied close to the origin of the pul monary artery and the heel of the clamp frequently encroaches on the anterior wall of the left or right branch of the main pulmonary artery Occasionally, where poststenotic dilation of the pulmonary artery is not great a smaller section of artery wall is occluded by the clamp Usually a 3 to 4 cm long incision is made in the clamped off portion of the anterior wall of the pulmonary artery A smaller incision is not satisfactory for a combined digital and instrumental valvulotomy A 5 to 6 cm long rubberized fabric diverticulum† is then sutured to the incision in the wall of the pulmonary artery If the incision is less than 4 cm in length the opening in the diverticulum must be sewn partially closed The suturing technique is illustrated in Figure 1 Essentially, an over and over suture of 4 0 arterial silk is placed and tied at either end of the incision These sutures are then continued on either side along the length of the incision Occasionally, one or more interrupted sutures are required to re enforce the continuous suture lines Purse string sutures of heavy (#2) silk are

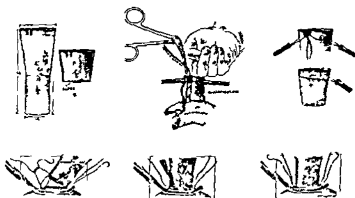


Fig 1

†Seamless Rubber Company New Haven Connecticut

then placed around the openings at the other end of the diverticulum and a catheter through which a dilute solution of heparin (20 mg /100 cm of normal saline) will be infused at the rate of 25 to 30 drops/min

With a clamp across the middle of the diverticulum the clamp on the pulmonary artery is slowly released If there is a free leak at the suture line of the diverticulum and the pulmonary artery the clamp is reapplied and extra sutures are taken as required If the suture line is water tight the clamp is removed from the pulmonary artery The operator then puts on thin gloves and inserts the index finger of the right hand into the largest opening in the diverticulum The purse string suture is tightened around the finger and the heparin infusion is begun The pulmonary valve is palpated directly and the position of the valve commissures and the position and depth of the sinuses of Valsalva are noted We have never palpated a valve orifice that was sufficiently large to admit the finger prior to valvulotomy The finger is withdrawn and the valvulotomy scissors or a Potts type valvulotome is inserted through the middle sized opening in the diverticulum The instrument can be guided into the stenosed opening by palpation from outside of the artery The initial incision is usually made into the anterior medial sinus of Valsalva When the incision made by the instrument is of sufficient size to admit the finger tip into the valve orifice it is usually easy to complete the valvulotomy by incisions deep into either two or three of the sinuses of Valsalva The finger is inserted into the right ventricle to determine the presence or absence of an infundibular stenosis Closure of the incision in the pulmonary artery is accomplished by a double continuous suture of 4 0 arterial silk after reapplication of the pulmonary artery clamp beneath the diverticulum and removal of the diverticulum The pericardial sac is left partially open to assure adequate drainage

Where the transventricular approach was used we employed the Potts modification of the Brock technique

RESULTS

Preoperative right heart catheterization was attempted in all cases but in 4 it was unsuccessful Postoperative catheterization was done in 15 patients but in one it was unsuccessful and in another only the pressure in the right ventricle was obtained Angiocardiography was carried out preoperatively in the majority of patients On the basis of the above studies and other observations an IASD or patent foramen ovale was suspected or proved in 19 cases and an IVSD in 3 cases Definite evidence of a right to left shunt was demonstrated preoperatively in 17 cases and suspected in 4 others Definite evidence of a left to right shunt was demonstrated in 3 cases and suspected in 2 others Symptoms referrable to the pulmonic stenosis was present in 29 patients prior to operation

Following operation one patient died This patient was 46 years old and died on the 6th postoperative day despite a successful TA valvulotomy Her case is referred to in more detail elsewhere[4] Three patients have been lost to followup Of the 12 patients with a TA valvulotomy followed for one year or more 1 still has some cardiac symptoms In this patient (C G Table 1) there was a heavy subvalvular band of muscle noted at

Table 1 Cardiac Catheterization Pulmonary Stenosis

PATIENT	AGE	PREOPERATIVE RV	PREOPERATIVE PA	POSTOPERATIVE VALUE MONTHS	POSTOPERATIVE VALUE RV	POSTOPERATIVE VALUE PA	PRESSURE GRADIENT RV PA PRE OPERATIVE	PRESSURE GRADIENT RV PA POST OPERATIVE
				A TRANSVENTRICULAR VALVULOTOMY				
S R	23	148/2	18/2	7½	70/0	24/3	130	46
L B	13	165/13		14	163/6	17/6		146
R B	12	173/0	5/0	13	120/0	13/6	168	107
B B	12	120/0	15/2	20	111/18	9/4	105	10[illegible]
D L	8	134/5	35/11	14	59/5	22/5	99	37
R C	11	150/3	17/2	24	52/14	12/6	133	40
				B TRANSARTERIAL VALVULOTOMY (DIVERTICULUM)				
A M	20	212/0	1/0	8½	96/7		211	
				36	35/	20/5		15
W C	19	118/0	20/9	7	58/1	30/8	98	28
				22	54/5	17/5		37
S I	20	94/0	10/3	6	22/1	22/9	81	0
C G	34	106/7	13/2	7	110/0	17/5	93	93
				16	78/0	7/2		71
P L	14	162/5	32/18	14	39/2		130	
G R	20	Unsuccessful		14	17/2			
D P	27	105/0	9/5	14	23/7	18/8	96	5
R C	18	154/6	1/0	18	42/2	27/7	153	15

operation and in the other patient a postvalvular constriction of the pulmonary artery was present Of the 14 patients with a TV valvulotomy 7 still have cardiac symptoms All of these are believed to be related to an inadequate valvulotomy, although in an associated infundibular stenosis cannot be ruled out

Postoperative catheterization studies when done (Table I) indicated a greater fall in the right ventricular pressures in the patients with the TA valvulotomy as compared with those having a TV valvulotomy The elevated pressure in the right ventricle in patient A M (Table I) eight months following TA valvulotomy *had returned to normal three years* following valvulotomy This patient was the first operated upon by this technique The persistently elevated pressures noted in C G (Table I) may be due to subvalvular muscular hypertrophy

Pulmonary insufficiency as evidenced by a diastolic murmur over the *pulmonary outflow tract was observed in 3* TA valvulotomy patients and in 2 TV valvulotomy patients It did not appear to be a clinically significant lesion in any of these patients

COMMENT

Transarterial pulmonary valvulotomy by a combined digital and instrumental approach is reserved for the older child or adult with pulmonary valvular stenosis Access to the pulmonary artery and right ventricle by the finger permits one to open with deliberation the stenosed pulmonary valve and to detect the presence of significant subvalvular or infundibular stenosis Return of the pressure in the right ventricle to normal after a satisfactory valvulotomy may occur at once or after several years, apparently depending on the extent of the subvalvular muscular hypertrophy In one additional patient, age 38, with a tetralogy of Fallot, both a subvalvular and infundibular stenosis were precisely relieved by the transarterial route

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AN EXPERIMENTAL SURGICAL TREATMENT FOR AORTIC INSUFFICIENCY*

BERNARDO CASTRO VILLAGRANA ALAIN SISTERON, AND MICHAEL E DE BAKEY

Creation of a new valvular mechanism is considered the ideal treatment for aortic insufficiency Two approaches to this problem have been attempted, namely, the use of a prosthetic valve and construction of a valve from the aortic wall itself

The former method has been employed by Hufnagel[1] using the ball valve principle and by Roe and his associates[2] using the flap-valve principle The latter method was used experimentally by Silen and his associates[4] and by Ryan and his associates,[3] and although *in some cases* a competent valve was obtained, in most instances partial stenosis, nonfunc

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tioning leaflets, or incompetency ultimately occurred While none of these methods has proved entirely satisfactory, there are reasons to believe that the latter approach to the problem may provide a better solution than that concerned with the use of a prosthetic valve Accordingly, this report is concerned with our investigations along these lines in *which a technique* has been developed for construction of a competent valve using the aortic intima

METHOD

Mongrel dogs weighing 9 to 18 kg were anesthetized with nembutal An incision was made through the fourth intercostal space, and the left subclavian artery and descending aorta were dissected to the first inter costal arteries

For creation of aortic insufficiency a simple crochet hook bent to a *suitable curve was introduced into the aorta through the subclavian artery*, hemostasis having been obtained by distal ligature of this artery and by a tourniquet placed around its origin A small opening in the pericardium permitted the left index finger to follow the progress of the hook, directed into the concavity of the posterior cusp of the aortic valve so that this cusp was perforated The force of withdrawal is sufficient to tear the entire cusp

Construction of the valve was performed at the segment of the aorta previously dissected, because it has no arterial branches After the first pair of intercostal arteries had been ligated, two rubber tourniquets were applied, one just below the subclavian artery and one just below the pair of ligated intercostal arteries A longitudinal incision of the aorta was then made, the opening having been maintained by two sutures of 5 0

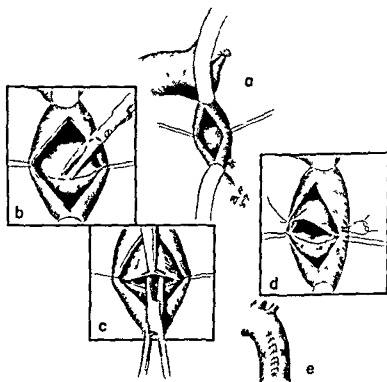


Fig 1 (a to e) Drawing illustrating technic of creation of new valve

arterial silk (Figure 1a) With fine sharp scissors the intima was sectioned transversely (Figure 1b), involving slightly more than one half the circumference in the region directly opposite the aortic incision The proximal edge of the intima so divided was lifted with smooth tissue forceps and separated from the media for a distance approximately one and one half times the diameter of the aorta (Figure 1c) This dissection must be rather wide and deep to assure an easy contact between the flap of the intima and the opposite wall in order to obtain a cusp similar to those of the aortic valve To keep the new valve in a functional position and to prevent further displacement of its free edge, both commissures were attached to their respective lateral aortic walls by mattress sutures in such a way that the free edge of the valve maintained a slight tension (Figure 1d) The incision of the aorta was closed with an over and over continuous suture (Figure 1e) The average time of occlusion of the aorta was 12 minutes The chest was closed in the usual manner, and penicillin was administered after operation

Blood pressures in the aorta, below the valve in all cases and proximal and distal to the valve in several, were recorded with a Statham strain gauge manometer and a Type D electroencephalograph These determinations were made during the operative procedures and periodically thereafter

Roentgenograms of the chest and retrograde angiography were performed in some cases to demonstrate aortic regurgitation, its repercussion over the heart, and the patency of the new valve

The 30 dogs were divided into three groups as follows 8 in which only construction of the valve was performed (Group 1), 4 in which aortic insufficiency was created without construction of the valve (Group 2), and 18 in which aortic insufficiency was created and a new valve constructed (Group 3), at the same operation in 15 and at an interval of 5 to 12 days in 3 dogs

RESULTS

None of the dogs in Group 1 died during the operation, and only one died of empyema during the postoperative period A thrombosed valve was encountered at autopsy The remainder were killed from one to 6 months after operation Pressure tracings on these dogs showed absence of stenosis at the level of the valve, although one case had an old thrombus, and the valves of dogs killed more than 4 months after operation were practically absent

Of the 4 dogs in Group 2, 3 died from 5 to 20 days after insufficiency was induced, and only one survived 2 months after destruction of the aortic valve The duration of life was inversely proportional to the extent of aortic regurgitation and its consequent enlarging of the heart The cause of death in all 4 was cardiac failure, and at postmortem examination the left ventricular wall was noted to be 3 to 4 cm thick

Of the remaining 18 dogs (Group 3) 8 died from 4 to 13 days after operation, 2 having had two operative procedures Cause of death in 5 was infection and in the other 3 death was attributable to traumatic lesions due to manipulation during destruction of the aortic valve Of the 10 dogs surviving more than 2 months, 6 were sacrificed at different periods between

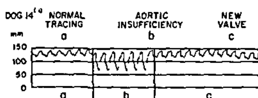


Fig 2 Blood pressure tracings showing in (a) normal curve (b) curve of aortic insufficiency and (c) curve after the construction of the new valve

70 and 260 days after operation, and the other 4 are still alive in good condition, one being a year postoperative

Valve construction was considered successful in 16 of the 18 dogs in this group Blood pressure tracings (Figure 2) showed that the differential pressure approximates its normal value with maintenance of a satisfactory diastolic pressure One of the 2 failures was doubtful because the degree of aortic regurgitation was minimal and therefore a judgment about the function of the valve was impossible

Periodic readings of blood pressure above and below the valve demonstrated no significant difference between systolic pressures proximal and distal to the valve, but if any exists it is almost invariably higher distally Diastolic pressure is 15 to 50 mm Hg higher distal to the valve than proximal At the same time, the diastolic pressure in the upper segment of the body, not receiving direct benefit from the valve, is higher than the previous diastolic pressure of the aortic insufficiency, ranging between 10 and 40 mm Hg more

No evidence of narrowing at the site of valve construction was found in the angiographic studies, and the existence of aortic insufficiency was clearly visualized by the filling of the left ventricle with opaque medium introduced through the carotid artery

Of the 14 specimens examined in this group of dogs, only one valve was thrombosed The remainder were all functioning with flexible and elastic cusps, and those older than 70 days had a well formed endothelium covering all inner surfaces of the valve and adjacent aorta The new valve contained an abundant amount of elastic tissue connected to the main portion of the aorta at the base of the cusp At the angle of separation between the valve and the aortic wall there was an area which showed evidence of fibrotic repair In all cases the destroyed cusp of the aortic valve which produced the aortic insufficiency was easily recognized, but the heart was not enlarged Nevertheless, although in two cases with aortic insufficiency previous to the construction of the valve a slight hypertrophy of the left ventricular wall was observed, in none of the dogs of this group was it more than 1 5 cm thick

COMMENT

The results of this study would seem to demonstrate the feasibility of construction of a competent valve using the above technique The valve does not produce stenosis and offers practically no resistance to systolic flow It is recognized that aortic lesions could limit the application of this procedure to human aortic insufficiency, however, in cadavers it was possible to construct competent valves under water pressure, even in aortas with advanced arteriosclerotic lesions It is also realized that a valve

located in the descending aorta cannot adequately correct aortic insufficiency, but these experiments are sufficiently encouraging to warrant application of the method to the ascending aorta and further investigation along these lines is in progress.

SUMMARY

A new technique is presented for creation of an aortic valve, utilizing the intima in such a way that a flap of it becomes a cusp similar to the cusps of the aortic valve Experimental results with this technique in the descending aorta, just below the subclavian artery, demonstrate a high degree of competence and maintenance of favorable histologic features.

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RIGHT HEART PRESSURE STUDIES AFTER VENTRICULOTOMY*

JAMES RAMS, HOWARD BRESLER, JOSEPH KISER, WILLIAM KISKEN, JAY WAGNER, AND PETER V. MOULDER

Open heart surgery for correction of intracardiac defects often requires a right ventriculotomy. This incision has not been innocuous Using hypothermia there is a much greater incidence of complications with ventriculotomy than with atriotomy or aortotomy,[1] in the presence of marked right ventricular hypertension it has been so troublesome that closure of ventricular septal defects has been suggested and performed through a right atrial approach to circumvent the ventriculotomy[2] Aneurysm formation and rupture have occurred but seem related to the thin walled infundibular chamber[3] It is interesting to note, however, that destruction of the outer right ventricular myocardium does not produce an elevated venous pressure nor right sided heart failure.[4,5,6] Muller *et al*[7] have studied the histopathology and tensile strength of right ventricle incisions in the dog and found no abnormalities.

*From the Department of Surgery of the University of Chicago Supported by grants from the Douglas Smith and Oscar Mayer Foundations for Medical Research

This is the initial phase of a series of dynamic studies on the effects of right ventriculotomy alone and demonstrates pressure effects and the sensitivity of these hearts to stimuli early and late postsurgery

METHOD

Adult mongrel dogs (10 to 25 kg) were given 20 to 30 mg morphine sulfate 1 to 2 hours prior to surgery Closed circuit ether-oxygen anesthesia was given via an intratracheal tube with a McKesson machine using manual bag compression during the open chest phase of the procedure Pressure records were made on a direct writer Grass polygraph with Statham P23D strain gauges A No 9 cardiac catheter† was employed for the closed chest measurements and a 20 gauge needle attached to the transducer by a 100 cm length of firm plastic tubing for the open chest procedures Mid right atrium was the zero level Femoral artery monitor of the systemic pressure accompanied these measurements Determinations were acceptable in steady state with nearly equivalent peripheral pressures

The incision in the right ventricular myocardium was made parallel to the anterior descending branch of the left coronary artery in the area relatively free of visible major coronary branches it was 4 to 6 cm in length roughly proportionate in the varying sized hearts The incision was made and progressively closed with finger pressure controlling hemorrhage to obviate an added unknown of circulatory occlusion oxygenator or hypothermia A continuous suture of 4 0 silk on an atraumatic needle was used At no time was a clamp applied to the myocardium

The first group of 8 animals had pressure measurements performed by catheterization and no specific cognizance was made of the proximity of the superior portion of the incision to the outflow segment of the ventricle The second group of 15 dogs had open chest measurements by direct needle puncture and the site of the incision relative to the outflow tract was carefully noted In this group pressure studies were performed after ventriculotomy after stabilization of the heart and blood pressure

RESULTS

In this laboratory the normal right ventricular and/or pulmonary artery systolic pressure at catheterization is 31 3 mm Hg (S D 7 2) with direct needle puncture and open chest it is 21 7 mm Hg (S D 7 1) Using simultaneous direct needle punctures of the pulmonary artery and right ventricle the differential of systolic pressures is 0 6 mm Hg (S D 1 27)

Group 1 (Catheterization Pressure Studies) Postoperative cardiac catheterization proved to be hazardous in this group of dogs often leading to fatal arrhythmias furthermore it made it impossible to acquire usable data in 10 dogs deleted from this series

Most observations were obtained in the right ventricle since more than a few easy attempts to move on into the pulmonary artery would lead to cardiac arrhythmia and deterioration of the animal

One dog showed no change in right heart systolic pressure (but was studied only in the immediate postoperative period) 3 developed an elevation 10 mm Hg in pressure but the final pressure was within the normal

† U S Catheter and Instrument Co

Table 1 Right Ventricular Pressures Via Cardiac Catheter (Closed Chest)

DOG	CONTROL	POST VENTRICULOTOMY PRESSURE	DURATION (DAYS)
290	35/0	75/0	8
		40/0	90
320	35/0	50/0	14
		22/10	180
260	21/0	32/0	5
		30/0	30
283	41/0	76/0	8
66	31/0	31/0	0
628	25/0	51/0	7
328	20/0	30/0	6
99	21/0	31/0	7

range, 4 revealed elevations of 20 to 35 mm Hg and these were well into the abnormal range

These elevations occurred within 1 to 2 weeks of surgery Late pressure recordings showed a return to the normal range in 60 to 90 days (Table 1) In only 2 animals was it possible to obtain pulmonary artery pressures and the pull through revealed a systolic pressure gradient (e g Dog 320, P A −19/9, R V −50/0) During catheterization a number of arrhythmias were produced and these were fatal in at least 2 dogs

Group 2 (Needle Puncture Pressure Studies — Open Chest). There are 12 satisfactory studies (Table 2, page 378) immediately post ventriculotomy which demonstrated (a) none had abnormally elevated pressures, (b) 7 showed virtually no change, (c) 2 had increases of pressure greater than 10 mm Hg, and (d) 3 had systolic pressure gradients across the pulmonary valve of 10 to 12 mm Hg

Seven studies from 6 to 24 days after ventriculotomy showed no abnormal pressures, no gradient across the valve and only one had an 18 mm Hg gain from the preliminary observation

As might be expected from the problems encountered with simple cardiac catheterizations there were severe complications and high mortality associated with reoperation on animals having had a right ventriculotomy In addition to the mortality from surgery the needle punctures often produced hazardous arrhythmias, eight of the 15 animals surviving for only 1 to 2 days and only one animal survived the rethoracotomy The general picture at post mortem examination was of acute heart failure, viz, a massively dilated right ventricle, engorged liver and pleural effusion Only one animal died of myocardial dehiscence due to massive infection The ventriculotomized dogs were markedly intolerant of rapid fluid infusions and some died with acute right ventricular dilatation

Table 2 Right Ventricular Pressures Via Direct Needle Puncture (Open Chest)

			POST VENTRICULOTOMY	
			LATE STUDIES	
DOG	CONTROL	AFTER STABILIZATION	PRESSURE	TIME (DAYS)
	mm Hg	mm Hg	mm Hg	
748	12/0	12/0	20/0	6
960	20/2		20/0	23
905	35/0	32/0	28/0	23
731	10/0	15/0	33/8	23
656	24/4	25/0	20/4	24
54	20/0	18/2	25/5	19
906	25/0	25/0	22/0	13
928	20/5	20/5		
822	17/1	30/2		
814	22/4	30/8		
821	10/0	35/10		
816	35/0	15/0		
818	25/0	20/0		
820	28/0	30/5		
297	30/2	34/4		

The marked sensitivity of the hearts of these animals producing deterioration of the animal during catheterization and surgery, or necessitating a short period of recording precluded acceptance of a considerable number of examinations as well as complete animal experiments for inclusion in the study

DISCUSSION

The ventricular hypertension found in some of the dogs studied by catheterization could be explained either on the basis of a functional and/or organic outflow obstruction or a reflex peripheral arterial spasm Since a systolic pressure gradient has been demonstrated the former would seem more likely Cardiac sensitivity has precluded settling this by catheterization

The studies in the second group of animals with direct puncture which were performed in anticipation of settling the question of outflow stenosis failed to give evidence of a marked pressure gradient furthermore no hypertension could be shown The low pressure and mild pressure gradient suggest a functional component to the stenosis These data may be inversely selective with animals having more hypertension (and stenosis?) succumbing readily

The findings of Rushmer[8] and others[9] of residual diastolic ventricular

volumes with an intact chest in contradistinction to the empty diastolic heart found with the chest open could help to explain this difference in effectiveness of the functional component of the outflow tract stenosis This would also help elucidate the delayed, sudden, 'unexplained' deaths seen after surgery on the abnormal right ventricle

Unquestionably there are differences in the pulmonary vascular resistance in these two situations that could explain the disparity in findings Immediately suggestive of such a difference is the variance in the normal right heart systolic pressures found at catheterization and direct study

Such an assessment of ventriculotomy *per se* indicates the magnitude of the insult in the normal dog heart and implies a greater problem in the abnormal, especially hypertensive right ventricle Such data should help to lead to therapy and help to evaluate attempts to develop new approaches to the ventricular chamber

CONCLUSION

These studies corroborate the impression that ventriculotomy alone is a significant insult to the heart to add to the deleterious effect of venous inflow occlusion, hypothermia, pump oxygenator and/or an intracardiac procedure

These findings are an occasional ventricular hypertension, a heart that is extremely sensitive to stimulation and stress, and a heart that will readily develop arrhythmias (often of a lethal character)

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EXPERIMENTAL STUDY OF THE EFFECTS OF TRANSECTION OF THE ANNULUS BY COMBINED ARTERIOVENTRICULAR INCISION FOR DIRECT SURGICAL REPAIR OF INFUNDIBULAR AND VALVULAR PULMONIC STENOSIS*

ROYCE E DAWSON, MICHAEL G WEIDNER, JR, AND
H WILLIAM SCOTT, JR

This study was instigated by an operating room experience in which the lack of knowledge concerning the effects of transection of the pulmonary annulus probably resulted in the loss of a patients' life due to the unsuccessful management of a valvular lesion with associated infundibular stenosis

METHOD

Ten healthy adult mongrel dogs of both sexes weighing from 9 to 14 kg were used in this study They were anesthetized with sodium pentobarbital (30 mg /kg) Cardiac catheterization was carried out measuring right atrial right ventricular, and pulmonary arterial pressures using Hathaway and Statham strain gauges and a Sanborn direct writing polyviso recording apparatus The animals were then immersed in an ice bath and their esophageal temperatures were lowered to 33 to 34°C Respirations were maintained by means of an endotracheal tube and intermittent positive pressure Bilateral anterior thoracotomy was carried out in the fourth interspace using sterile technique The azygos vein was ligated and tapes were placed around the superior and inferior vena cavae The pericardium was then opened longitudinally anterior to the phrenic nerve The area of the junction of the superior vena cava and the right atrium was infiltrated with 1% xylocaine Traction sutures of 4 0 arterial silk were then placed anteriorly in the pulmonary artery and right ventricle approximately 2 cm proximal and distal to the pulmonic annulus After inflow stasis had been achieved by traction on the tapes around the cavae, an incision was made between the traction sutures which completely opened the pulmonary outflow tract (Fig 1) No attempt was made to avoid the valve cusps and the annulus was completely divided The pulmonary valve cusps were partially excised in 2 animals The incision was closed with a continuous 5 0 arterial

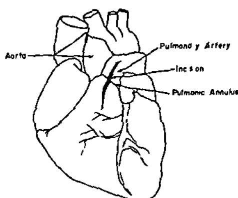


Fig 1 Drawing of heart showing line of incision

*From the Department of Surgery Vanderbilt University School of Medicine Nashville
Supported by a grant from the Middle Tennessee Heart Association

silk suture and inflow stasis was released The period of inflow stasis varied from 2 to 5 minutes The thoracotomy incision was then closed and the animals were rewarmed slowly in a warm water bath

The dogs were kept in cages and fed a standard kennel ration for periods varying from 9 to 14 months For 3 weeks prior to recatheterization the animals were allowed to run free in the kennel for 3 to 4 hours per day Cardiac catheterization was again carried out just before sacrifice (9 to 14 months postop) and pressures recorded as before

At autopsy in each animal the heart was examined and studies of heart weight were carried out as described by Herrmann [2]

RESULTS

After recovery from operation the animals remained in good general condition and showed no ill effects from the procedure Examination prior to sacrifice and after the period of exercise revealed no evidence of right heart failure in any animal

A summary of catheterization data is seen in Table 1 A comparison of preoperative and postoperative catheterization studies reveals that there was no rise in the right auricular pressures in the animals with one exception where a rise of 8 mm /Hg mean pressure was recorded In the latter animal a healed infarct was found in the anterior wall of the right ventricle

A study of the postoperative pressures in the right ventricle reveals an insignificant increase in two instances and a slight lowering in all others The right ventricular pressures were all within the normal range There was no rise in the mean pressures or the end-diastolic pressures

Table 1 Pressures in mm /Hg Recorded at Cardiac Catheterization

DOG NO		RIGHT AURICLE	MEAN	RIGHT VENTRICLE	MEAN	PULMONARY ARTERY	MEAN
310A	Preop	8/3	5	51/3 8	25	53/36	42
	Postop	7/0	2	45/0 6	19	19/4	16
487A	Preop	8/6				12/8	11
	Postop	7/4	5	23/0 2	11	16/4	9
557A	Preop	8/5	5	37/6	20	43/30	37
	Postop	5/1	2	42/0 3	19	33/4	22
564A	Preop	6/2	4	28/0-4	13	22/9	15
	Postop	6/0	2	18/0 2	8	16/6	10
597A	Preop	7/3	4	31/3-4	20	25/14	20
	Postop	4/2	2	22/0-4	10	20/8	12
613A	Preop	13/8	10	35/0 5	18	32/14	19
	Postop	3/0	1	32/0 2	14	30/10	16
628A	Preop	11/2 7	5 4	42/0 8	25	48/10	25
	Postop	3/1	2	32/0 6	16	33/7	20
693A	Preop	17/12	14	21/0 2		22/9	14
	Postop	6/2	2	32/0	11	22/8	12
695A	Preop	10/0	2	40/0 6	23	44/21	29
	Postop	12/9	10	39/0 6	22	46/10	29
696A	Preop	5/0	3	39/0 2	14	26/12	17
	Postop	4/0	1	29/0 3	12	26/10	16

A significant fall in pulmonary artery pressure was noted in or animal The mean pressure gradient across the pulmonary valve sh significant change in only 2 animals (310A and 557A) and it sho noted that these 2 animals both had a high pressure gradient preopei which returned to normal levels after operation It should also b tioned that these 2 animals both had portions of their pulmonary cups excised at the time of operation The highest mean pressure g across the valve postoperatively was 7 mm /Hg

Postmortem examination of these animals revealed no evidence o heart failure The valve cusps were found to be partially exc animals No 310A and 557A One valve cusp was noted to be spl additional animals There was no evidence of right ventricular trophy or dilatation in any of the animals and no evidence of s was seen in the region of the annulus An old healed myocardial was observed in the anterior wall of the right ventricle in animal No

The average of the ratios of the fresh ventricular weight to body was found to be 00718 with a low of 00619 and a high of 00954 series of 200 normal dogs Herrmann[2] found the average ratio of ventricular weight to body weight to be 00789 with a low of 0051 a high of 00905 In Figure 2 one sees the ventricular weight plotted a the body weight on log log graph paper Here it is noted that th of the experimental animals fall within the group that Herrmann co as normal

DISCUSSION

Theoretically several abnormal postoperative findings were antici damage to pulmonary valves, stenosis, pulmonic insufficiency, and heart failure Damage to the pulmonary valves did occur but w evidence of physiologic difficulty Neither pulmonic valvular nor a stenosis was demonstrable either physiologically or anatomically was no significant demonstrable pulmonic insufficiency No evide right heart failure was found

The catheterization data and heart weight to body weight ratios to indicate that there is no significant discernible damage to the pulm outflow tract by incision through the annulus The 2 animals in the valve cusps were partially excised seemed to tolerate this damage as one would expect from the work of Ratcliffe *et al*[5] and Kay[3] damage to valves in other animals was tolerated well

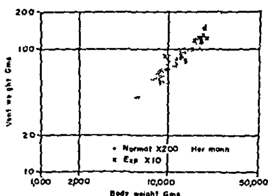


Fig 2 Fresh ventricular weights against body weights on log log paper The dots represent the norm of Herrmann[2] and the xs the experi animals

Lillehei[4] and Cooley[1] have successfully used this type of incision in conjunction with extracorporeal circulation Although this incision is not advocated for routine use in correction of infundibular stenosis, it may be of value in dealing with combined valvular and infundibular lesions No serious sequellae have been demonstrated by its experimental use

SUMMARY AND CONCLUSIONS

A study has been made of the physiologic and pathologic changes that occur following opening the entire outflow tract of the right ventricle with transection of the pulmonary annulus Under the conditions of this experiment, no ill effects of transection of the annulus were observed Such an incision would permit the operator to view and deal with the whole outflow tract of the right ventricle when necessary and should be more applicable in conjunction with extracorporeal circulation

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PERICARDIAL VALVE GRAFTS IN THE SURGICAL THERAPY OF MITRAL INSUFFICIENCY*

ALVIN A BAKST AND LEO LOEWE

Severe rheumatic mitral insufficiency seems to be due to two related factors (1) a deficiency in valve substance of the mural leaflet at the posterior commissure, and (2) a dilatation of the annulus fibrosis Apparently, these two factors are intimately related the dilatation of the annulus being secondary to an initial deficiency in valve substance

Utilizing this concept, the authors have attempted to create, and surgically correct this lesion by the addition of a pericardial valve graft to the posterior half of the mural leaflet of the mitral valve

METHOD

Mongrel dogs were anesthetized with intravenous sodium pentothal,

*From the Division of Thoracic Surgery and the Udo M Reinach Cardio Pulmonary Laboratory The Jewish Hospital of Brooklyn Supported by a grant from the New York Heart Association

after which a left thoracotomy incision was performed through the fifth intercostal space Mitral regurgitation was created by resecting the posterior portion of the mural leaflet with a mastoid rongeur, after which the chordae in this region were avulsed with a nerve hook

Initially, an attempt was made to prepare a series of animals with chronic mitral insufficiency However, since the creation of a significant regurgitant lesion was invariably attended by a mortality due to pulmonary edema within 12 to 24 hours, the necessity for the immediate correction of the lesion became apparent The present experiment, therefore, comprises a series of animals in which the creation of a significant mitral insufficiency was followed by the immediate insertion of a pericardial valve graft for its correction Intracardiac pressures from the left atrium and aorta, and an electrocardiogram were secured before and after the creation of the mitral regurgitation, and again after its surgical correction

Technique of Repair of Mitral Insufficiency. A pericardial valve graft is first prepared for insertion A rectangular segment of pericardium, measuring approximately 4 by 7 cm, is secured Tails are prepared so that the completed valve may be anchored along both its apex and base A piece of muscle is incorporated into the valve graft to provide the bulk and substance necessary to tamponade the regurgitant jet Upon completion, a valve graft is created which can be anchored at multiple points along its base, as well as at its apex The anchorage of the apex into the posterior papillary muscle serves as a chorda tendinea The bare left index finger is inserted into the atrium, and the posterior commissure is located The first suture† is placed to the right of the posterior descending artery, into the left ventricle just beneath the posterior commissure The suture then passes between the valve leaflets into the left atrium, and out through the auricular appendage The end of the suture is immediately attached to the corresponding tail of the valve graft The next suture is placed in the mural leaflet anterior to the regurgitant jet, at a point approximately midway between the two commissures This suture is passed from outside the heart just below the coronary vessels, and is immediately angled upwards to enter the left atrium at the junction of the valve leaflet with the annulus This suture, too, is attached to the corresponding tail of the graft A third suture is similarly placed midway between the first two The finger is then placed into the left ventricle against the posterior papillary muscle A suture is passed into the left ventricle at this site, and is guided between the valve leaflets into the atrium It is withdrawn from the auricular appendage, and attached to the tail of the valve graft (Fig 1) The pericardial valve graft is inserted into the atrium and is snugged into its proper position by pulling on the appropriate sutures The tails are then anchored to the ventricular myocardium

In this fashion, a valve graft placed across the posterior portion of the mitral orifice can replace a portion of a resected leaflet of the mitral valve (Fig 2) The bulkiness of the graft immediately tamponades the regurgitant jet without in any way interfering with the mobility of the aortic leaflet

†Prepared by J A Deknatel & Son Inc, Queens Village, Long Island New York

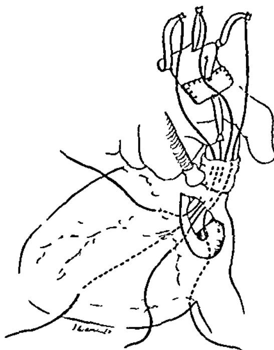
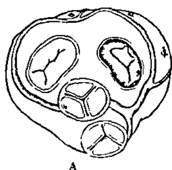


Fig 1 The first suture is placed posteriorly into the left atrium just beneath the posterior commissure, between the valve leaflets and out through the auricular appendage The second suture is placed anterior to the regurgitant jet, from below the coronary vessels exteriorly, to the junction of the leaflet with the annulus internally The third suture is similarly placed midway between the first two The fourth suture is passed into the posterior papillary muscle for placement of the graft chorda The sutures are attached to the respective tails of the graft

(From Journal Thoracic Surgery, in press)



A



B

Fig 2 (a) The pericardial graft is inserted into the atrium and is snugged into position by pulling on the appropriate sutures thereby tamponading the regurgitant jet
(b) Same, with heart open demonstrating the final placement of the tails of the graft into the valve leaflet and papillary muscle

(From Journal Thoracic Surgery, in press)

RESULTS

Physiologic Observations. In all animals, after insufficiency was produced, a typical regurgitant atrial pressure tracing was observed There was an elevation of the atrial C wave After the valve graft was inserted, the regurgitant jet was abolished, with reversion of the atrial pressure tracing to normal

Pathologic Observations. In 4 animals, 2 of which were sacrificed at 6 and 8 months respectively, no regurgitation existed at time of sacrifice In each, the graft had become adherent to the mitral valve, and completely replaced and resected portion of the leaflet The base of the graft was entirely adherent to the annular attachment of the valve The apical

chorda was thickened and fibrotic The graft seemed to have completely and effectively replaced the resected portion of the valve In one animal sacrificed after 6 months, although the major regurgitant jet had been abolished, a residual fine jet could be palpated In this animal the valve graft was found to be in proper position, but the base was adherent only at its points of fixation The regurgitant jet palpated prior to sacrifice, had emerged between the points of fixation

One animal died after 5 months, of peripheral embolization Postmortem examination revealed the base of the graft to have been avulsed at two points of fixation The pericardial graft was found within the ventricular chamber, and surrounded by old and recent thrombus

DISCUSSION

Bailey[1] believes that normally, although there is a considerable excess of valve leaflet for coaptation anteriorly, there is very little such tissue posteriorly This relationship is seriously altered by the thickening, rolling and shortening of the valve leaflets and chordae, caused by the rheumatic infection The anterior portions of both the mural and septal leaflets, having an excess of value tissue, seem to tolerate this shortening without loss of competence The posterior portions of these leaflets, however, barely coapting in the normal state, are rendered incompetent by this pathologic process In truth, there seems to be an actual deficiency of valvular substance of the posterior aspect of the mural leaflet, which may be so severe that only a residual rim of atrophic tissue remains During cardiotomy for predominant mitral stenosis associated with insufficiency, it has been recognized that the regurgitant jet almost invariably emerges through the posterior commissure Proper placement of the exploring finger in this region can effectively tamponade the regurgitant jet, as evidenced by a rise in systemic pressure, and an increase in the fullness of the peripheral pulse This fact, coupled with the pathologic finding of an absolute deficiency of valvular substance of the posterior portion of the mural leaflet, has led to the concept of tamponading the regurgitation by the proper placement of a valve graft which would adhere to, and add substance to the atrophic portion of the mural leaflet This valve substance must tamponade the regurgitant flow of blood by enabling the posterior portion of the aortic leaflet to coapt against it during ventricular systole

Experience has demonstrated that the mobility of the aortic or septal leaflet cannot be impaired

The operation for insertion of a valve graft is simple, and permits the utilization of a closed technique The diagonal placement of the graft prevents the future development of a stenosis of the orifice by, at all times, insuring full mobility of the aortic leaflet In all animals the graft is incorporated into the substance of the mural leaflet

Since the advent of open heart surgery, the posterior commissure of the mitral valve can be sutured directly with immediate tamponading of the regurgitant jet In our clinic such a direct repair of the regurgitant portion of the mitral valve has been accomplished in two patients

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SURGICAL HEALING OF THE ATRIOVENTRICULAR LEAFLETS AN EXPERIMENTAL STUDY*

WILLIAM S LYONS AND JOHN W KIRKLIN

References to the ability of cardiac leaflet tissue to withstand direct surgical incision and suture are few Shumway and Lewis[1] have reported that sutures are poorly tolerated in the aortic leaflet of the left atrioventricular valve in the dog, and that in animals which survived 2 months there was scarring and calcific deposit in the region of the suture Robicsek[2] has recorded fairly regular healing of leaflet to annulus in homografts of the septal leaflet of the right atrioventricular valve of the dog, although it is not clear whether in fact a portion of the original basal attachment had not been left attached to the grafts Riberi and associates[3] and Kay, Kaiser and Gaertner[4] have obtained regular firm fusion from suture of the commissures of the pulmonary valve leaflets

The present study was undertaken to observe the healing properties of the tissue of both right and left atrioventricular leaflets proper in response to direct incision and suture

METHOD AND RESULTS

All studies were done under direct vision employing hypothermia, inflow stasis, and open cardiotomy Continuous 5 0 arterial silk was used for all suture lines The animals were sacrificed 3 to 7 weeks postoperatively, with the exception of 1 dog which died 24 hours after operation

Mongrel shepherd dogs weighing 11 to 22 kg were utilized Pentobarbital sodium (nembutal) in an average dose of 35 mg/kg of body weight provided the anesthesia The animals were clipped to the skin, intubated, and immersed in tap water at 11° to 15°C Throughout the period of cooling, respiration was maintained artificially at the rate of 20 to 30 cycles per minute by use of an open circuit mechanical respirator and 100% oxygen When the rectal temperature reached 32°C the animals were removed from the bath and dried Hyperventilation at 40 to 50 respirations per minute was employed for 30 minutes preceding and following inflow occlusion The approach to the atria was through the fifth right or left intercostal spaces With a right thoracotomy, tapes were placed about the venae cavae and the azygos vein was tied With a left thoracotomy inflow occlusion was accomplished in the manner described by Shumway and Lewis[1] by passing a single umbilical tape through the transverse sinus behind the right atrium, anterior to the cavae and under the heart Ventricular fibrillation was a frequent occurrence during the period of occlusion and was deliberately provoked during procedures on the left side of the heart by striking the septum with a needle holder Before the re-establishment of circulation the heart was filled by release of the tapes Defibrillation was readily accomplished with massage, epinephrine, and electric shock The useful operating time provided by these methods was 8 minutes

Right Atrioventricular Valve. Eighteen dogs were employed for procedures on the right atrioventricular (tricuspid) valve

*From the Mayo Foundation and the Mayo Clinic Rochester Minnesota



Fig 1 The right atrioventricular (tricuspid) valve in a dog *a* Arrows indicate the healed incision in the septal leaflet 24 days after operation *b* Arrows indicate the surgically closed and healed anterior commissure 21 days after operation (Note in figure 1*a* the normal appearance of the anterior commissure)

Incision of Leaflet In 10 animals the septal leaflet was incised from basal attachment to free margin and immediately resutured The incision healed in all cases and there were no fenestrations or dehiscences at the suture line Edema and hemorrhage were still present in the suture line of one animal 3 weeks after operation, however, the inflammatory reaction had subsided in all the other animals sacrificed at this time A firmly adherent 5 mm thrombus was present on the atrial surface of the otherwise well healed suture line in one animal In 5 there was mild to moderate contracture of the suture line which resulted in only minor degrees of distortion of the leaflet In 3 animals healing was complete and free of thrombus or deformity (Fig 1a)

Suture of Commissure In an additional 7 of the 18 dogs the anterior and septal leaflets of the tricuspid valve were sewn together along their free margins at the intervening commissure This was difficult to accomplish satisfactorily because of the delicate and diaphanous structure of the anterior leaflet However, 5 of the 7 suture lines united in a manner that satisfactorily demonstrated the ability of the unfreshened edges to heal despite the rapid movements and fluctuations of pressure to which the leaflets are subjected Three showed small fenestrations comprising less than 20% of the suture line and 2 healed optimally (Fig 1b) No union of the commissure was accomplished in 2 animals

Transection of Leaflet In the final animal of the group of 18 the septal leaflet of the right atrioventricular valve was completely transected on a line approximately 3 mm from and parallel to its basal attachment and the cut was resutured immediately Healing was satisfactory with minimal scarring

Left Atrioventricular Valve. In 9 dogs the aortic leaflet of the left atrioventricular (mitral) valve was incised from its basal attachment to the free margin and immediately resutured In 5 of these the leaflet healed without dehiscence or deformity In the other 4 moderate to large dehiscence of the suture line occurred The lesions were found immediately adjacent to the basal attachment of the leaflet they did not extend into the appositional edges It was impossible to determine whether the dehiscence was



Fig 2 The atrial surface of the aortic leaflet of the left atrioventricular (mitral) valve of a dog Arrows indicate the healed incision in the leaflet 22 days after operation

due primarily to breaking of the silk or to tearing of the leaflet substance by the suture The latter occurrence was suggested in one animal which died 24 hours following operation and in which the edges of the incision were clearly serrated In 2 of the 5 dogs in which solid healing occurred, a firmly adherent thrombus 6 to 7 mm in diameter was present on the atrial surface of the suture line The ventricular or outflow surfaces however were smooth and devoid of thrombus In the remaining 3 dogs, the suture line was smooth there was little scar tissue and no contracture (Fig 2)

It is noteworthy that a slight change of technique appeared to influence the results favorably The 4 dogs in which dehiscence was found were among the first 5 to undergo operation In the remaining 4 dogs the sutures were placed closer together and were made to include a broader portion of leaflet tissue With this modification all 4 healed satisfactorily

CONCLUSIONS

In view of the small number of animals used in this study the exact relative frequency of the various results has little significance The important findings are

1 The valve leaflets after direct surgical attack can display a structure close to normal without significant atrophy

2 Suture lines in this delicate tissue can be made to hold with regularity on the right side of the heart where stresses are lower, and likewise even amid the higher stresses of the left side

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TRANSBRONCHIAL LEFT HEART CATHETERIZATION A MODIFIED TECHNIQUE AND ITS PHYSIOLOGIC EVALUATION*

ANDREW G MORROW, EUGENE BRAUNWALD, AND HERBERT L TANENBAUM

Left heart catheterization at the present time is usually performed by either the posterior percutaneous or the transbronchial route The chief advantage of the transbronchial method of left atrial puncture is its safety, which has been proved in a series of more than 700 left heart catheterizations carried out at the National Heart Institute without a death or serious complication [1] In the past, however, the application of transbronchial left heart catheterization in clinical physiological studies was limited The presence of the bronchoscope precluded measurement of oxygen consumption and the application of the direct Fick method for the determination of cardiac output at the time of left heart pressure measurements Bronchoscopy itself served as a physiologic stress, the magnitude of which was unknown and which appeared to vary from patient to patient Thus hemodynamic measurements could not be made with the patient in a steady basal condition or under a known degree of stress Furthermore, it was not possible with the original technique to measure left atrial and left ventricular pressures simultaneously and exercise studies could not be conveniently carried out

METHOD

These limitations have been obviated by recent modifications in technique After suitable premedication and with topical anesthesia a bronchoscope is passed into the first portion of the left main bronchus and a specially designed needle† is inserted anteriorly into the left atrium A polyethylene catheter is then threaded through the needle, across the mitral valve and into the left ventricle After the measurement of the pressures in these chambers and in a systemic artery or the aorta, cardiac output is determined by the injection of indicator dye into the left ventricle Arterial blood is constantly withdrawn through a cuvette densitometer[2] by a motor driven syringe and the dye dilution curve is recorded and subsequently calibrated [3] The catheter is then temporarily disconnected from the pressure transducer and the transbronchial needle withdrawn over it while the catheter tip remains in the left ventricle For simultaneous measurements of left atrial and left ventricular pressures a

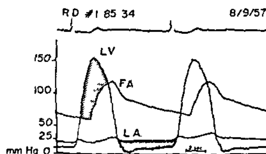


Fig 1 Pressures recorded simultaneously from the left atrium left ventricle and femoral artery in a patient with combined mitral and aortic stenosis The stippled area shows the mitral valve filling pressure gradient and the cross hatched area the aortic valve gradient

†Manufactured by the Becton Dickinson Co Rutherford N J

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second transbronchial puncture is made (Fig 1) The bronchoscope and needle are then removed, the catheter still remaining *in situ*

The patient rests quietly for 20 to 30 minutes after which oxygen consumption is measured by the collection and analysis (Scholander) of expired gas A second series of hemodynamic observations is then carried out with repeat pressure measurements and determination of cardiac output by indicator dilution The effects of exercise or of various drugs on left heart pressures may then be observed for prolonged periods

In 10 patients duplicate determinations of resting oxygen consumption were made at the time of right heart catheterization and the average value was compared to the oxygen consumption measured in the course of subsequent left heart catheterization Figure 2 illustrates that in all but 2 patients the results compared closely and that a basal state could be readily achieved This was further confirmed by the basal respiratory quotients and ventilatory volumes observed 20 to 30 minutes after the bronchoscope had been removed

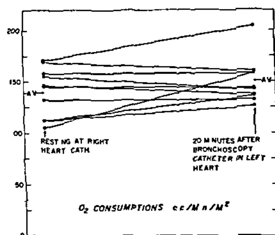


Fig 2 Determinations of oxygen consumption during right heart catheterization and left heart catheterization in the same patients Right heart values are the average of two determinations Left heart values are those measured after bronchoscopy with the catheter remaining in the left atrium or left ventricle

Table 1 Comparison of Cardiac Indices[1] at Left and Right Heart Catheterizations in Patients with Valvular Heart Disease

PATIENT	CARDIAC INDEX AT RIGHT HEART CATHETERIZATION CATHETER IN PULMONARY ARTERY: REST[2]	EXERCISE	INCREASE IN O_2 CONSUMPTION WITH EXERCISE cc./min /M²	CARDIAC INDEX AT LEFT HEART CATHETERIZATION: AFTER BRONCHOSCOPY CATHETER IN LEFT HEART	BRONCHOSCOPE IN PLACE
W M	1 66	3 46	259	1 69	2 09
J M	1 89	4.58	298	1 82	2 01
A G	2 80	3 96	183	3 04	2 60
A R	2.89	3.21	101	2 77	3 87
F D	3 14	4.55	285	2.25	2.24
F B	3.26	5.57	248	1.90	2 16
D.S	4.27	7 74	228	1 97	2.50
M F	4 47	5 06	242	2.61	3.21

[1] Cardiac index = Cardiac output in L./min /M²

[2] Average of two determinations

In 8 patients determinations of cardiac output were made by the Fick method at the time of right heart catheterization at rest and during the 8th to 10th minutes of exercise on a bicycle ergometer These results are compared in Table 1, with cardiac output determined at left heart catheterization on another day, with the bronchoscope in place and 20 to 30 minutes following its removal In 4 patients the resting cardiac indices during right and left heart catheterization compare quite closely and are within 0 25 L /min /M ² of each other In the other 4 patients the resting output at left heart catheterization was substantially lower than that measured at right heart catheterization When the bronchoscope was in place the cardiac index averaged only 0 33 L /min /M ² higher than the subsequent resting value In contrast when the same patients were subjected to exercise on a bicycle ergometer resulting in an average increase in oxygen consumption of 230 cc /min /M the average increase in cardiac index was 1 72 L /min /M ² Thus in most patients bronchoscopy did not result in a substantial rise in cardiac output

DISCUSSION

The modified technique described has been applied in a variety of clinical hemodynamic studies It has made possible more precise characterization of stenotic mitral and aortic valvular lesions since flow measurements can be combined with the determination of pressure gradients and the effective orifice size can be estimated [4] The ability to extend the duration of left heart pressure measurements has for example permitted the detection of mitral insufficiency even in the presence of associated mitral stenosis During norepinephrine infusion the elevation of systemic resistance increases the mitral regurgitant volume and markedly elevates the left atrial v wave In the absence of mitral insufficiency norepinephrine infusion has been found to have relatively little effect on the left atrial pressure pulse contour [5]

CONCLUSIONS

On the basis of the data presented the transbronchial method of left heart catheterization appears preferable to the percutaneous one since it combines the advantages of safety and the ability to make extended hemodynamic measurements with the patient in a known physiologic state

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C Problems in the Physiology of Extracorporeal Circulation of the Heart and Vascular Grafts

STUDIES OF ACID BASE DERANGEMENT DURING TOTAL CARDIAC BYPASS*

ROBERT G PONTIUS, ELTON WATKINS, BRUCE S MANHEIM, ROBERT G ALLEN, LESTER R SAUVAGE AND ROBERT E GROSS

One of the problems associated with the use of a pump-oxygenator for extracorporeal circulation is that of the development of metabolic acidosis during perfusion Studies of acid base derangement were undertaken in an attempt to relate metabolic acidosis to anesthesia, perfusion rates, arterial anoxemia, and variations in arterial blood carbon dioxide tension

METHOD

The 79 mongrel dogs studied during total body perfusion ranged from 6 to 66 kg in weight Anesthesia was induced with pentothal and main tained with cyclopropane and oxygen by means of a manually controlled closed circuit During the period of bypass the lungs were held gently inflated with a mixture of helium and oxygen (80% to 20%) Right thora cotomy was performed Large cannulae were placed in the *cavae*, right atrium, and left subclavian artery In animals with low flow rates, the perfusion times were 20 minutes in animals with the high flow rates, the perfusion time varied from 20 minutes to 2 hours, but generally were one hour During the bypass, various manipulations were carried out within the heart

Pump Oxygenator Different types of pumps and oxygenators were used during these experiments There did not appear to be any direct relation ship between the variation in equipment used and the derangements found in acid base balance Our present oxygenator is a rotating disc Kay Cross type and is used with a DeBakey type pump

Method of Analysis of Acid Base Derangement Samples of arterial blood were drawn at specific times during the procedure Plasma pH was promptly determined on whole blood, using an anaerobic glass electrode assembly (Beckman) at 37°C Oxygen and carbon dioxide content of the blood was determined using the Van Slyke apparatus Hematocrit deter minations were made in Wintrobe tubes Carbon dioxide tension and buffer base concentrations were obtained using the nomogram of Singer

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*From the Surgical Research Laboratory of The Children's Hospital and the Depart ment of Surgery of the Harvard Medical School Boston Massachusetts Aided by grants from the National Heart Institute of the Public Health Service and by a grant from the American Heart Association

With the technical assistance of Cynthia G Letteney R N Virginia K Vogel and Betty L Almond R N

and Hastings[7] The buffer base is that concentration of bicarbonate and hemoglobin anions which can neutralize acids added to whole blood Metabolic acidosis is indicated by a decrease in buffer base (which is measured in mEq /L) The carbon dioxide tension in the blood was controlled directly by ventilation of the lungs prior to bypass, and by appropriate gas mixtures for the oxygenator during perfusion The mixture was usually 2% carbon dioxide and 98% oxygen Anesthesia, perfusion rate arterial blood oxygenation and carbon dioxide tension were controllable variables, while the concentration of buffer base was the observed variable Mean values for groups of observations are tabulated

Influence of Anesthesia and Thoracotomy. Metabolic acidosis developed consistently in 29 observations between the induction of anesthesia and the time for beginning the perfusion During this time the average pH value fell from 7 41 to 7 32 and the buffer base value fell from 45 1 mEq /I to 40 0 mEq /L Such a development of metabolic acidosis with anesthesia and thoracotomy has been reported by others[1]

Table 1 Effects of Anesthesia and Thoracotomy Prior to Bypass

ANIMALS STUDIED	BEFORE ANESTHESIA pH	pCO₂ mm Hg	BB mEq /L	AFTER CANNULATION pH	pCO mm Hg	BB mEq /I	CHANGES pH	pCO₂ mm Hg	BB mEq /L
29	7 41	32 3	45 1	7 32	36 5	40 0	—09	4.2	—5 1

Influence of Perfusion Rate. Metabolic acidosis progressed during the period of bypass The degree of progression was more severe with lower perfusion rates A fall in buffer base of 6 4 mEq /L at a flow rate 20 to 30 cc /kg /min is to be compared with a fall of only 1 6 mEq /L at a flow rate of 50 to 60 cc /kg /min Statistical analyses revealed significant differences in acidosis between the group with a flow rate 20 to 30 cc /kg /min and those groups with flow rates above 40 cc /kg /min

Table 2 Effect of Perfusion Rate During Bypass

FLOW RATE cc /kg /min	ANIMALS STUDIED	BEFORE PERFUSION pH	pCO₂ mm Hg	BB mEq /L	END OF PERFUSION pH	pCO₂ mm Hg	BB mEq /L	CHANGES pH	pCO₂ mm Hg	BB mEq /L
20 30	7	7 33	31 0	38 5	7 18	31 6	32 1	— 15	+0 6	—6 4
30-40	8	7 37	33 2	42 9	7 24	38 1	37 6	— 14	+4 9	—5 3
40 50	7	7.28	36 4	39 7	7.25	41 0	37 7	— 03	+4 6	—2 0
50 60	9	7.33	35 6	40.8	7.27	35 0	39 2	— 06	—0 6	—1 6
60 140	42	7 31	35 0	39 8	7.25	34 9	37 7	— 06	—0 1	—2 1

Influence of Incomplete Oxygenation of Arterial Blood. In a group of experiments arterial oxyhemoglobin saturations were kept below 80% while perfusion rates were 40 to 60 cc /kg /min Metabolic acidosis devel

oped to a level of 4.8 mEq./L. and was significantly greater than the value of 1.8 mEq./L. in a similar perfusion rate group which had arterial saturation above 80%.

Table 3. Effect of Incomplete Oxygenation of Arterial Blood

ART. OX. SAT'D	ANIMALS STUDIED	BEFORE PERFUSION pH	pCO₂ mm. Hg	BB mEq./L.	END OF PERFUSION pH	pCO₂ mm. Hg	BB mEq./L.	CHANGES pH	pCO₂ mm. Hg	BB mEq./L.
Over 80%	16	7.31	35.2	40.5	7.26	37.6	38.7	—05	+2.4	—1.8
Under 80%	6	7.38	27.7	40.5	7.22	45.9	35.7	—16	+18.2	—4.8

Influence of Hypocapnia. Reduction of the tension of carbon dioxide (pCO_2) in the blood below a normal range of 35 to 45 mm. Hg produces an elevation in plasma pH. For purposes of analysis a pCO_2 value below 30 mm. Hg was considered to be hypocapnia. Forty-two animals with flow rates between 60 and 140 cc./kg./min. were divided into 4 groups: (A), those in which hypocapnia was never present, (B), those in which hypocapnia was induced during perfusion, (C), those in which hypocapnia was present before and throughout perfusion, (D), those in which hypocapnia was present before perfusion but was abolished during perfusion (Table 4).

Table 4. Effect of Hypocapnia Before and at End of Perfusion

GROUP	ANIMALS STUDIED	HYPOCAPNIA BEFORE	HYPOCAPNIA AT END	BEFORE PERFUSION pH	pCO₂ mm. Hg	BB mEq./L.	END OF PERFUSION pH	pCO₂ mm. Hg	BB mEq./L.	CHANGES pH	pCO₂ mm. Hg	BB mEq./L.
A	20	0	0	7.28	40.8	40.8	7.23	41.6	38.2	—05	+8	—2.6
B	6	0	+	7.15	52.2	39.0	7.29	26.9	37.8	+14	—25.3	—1.2
C	7	+	+	7.44	24.0	42.1	7.31	21.9	35.7	—13	—2.1	—6.4
D	9	+	0	7.38	19.3	35.8	7.24	35.6	37.5	—14	+16.3	+1.7

In group C where hypocapnia was present both at induction and termination of perfusion a drop in buffer base of 6.4 mEq./L. indicated a striking progression of metabolic acidosis. In group D, where hypocapnia was present at the beginning of perfusion but abolished during perfusion, there was an increase in buffer base of 1.7 mEq./L. Comparison of the buffer base changes in Group C with other groups was significant. Changes in the plasma electrolyte pattern (Table 5) in an animal with severe hypocapnia from Group C suggests that the magnitude of sodium and bicarbonate shifts are greater than those estimated for the compartment which contains acid radicals.

Table 5 Plasma Electrolyte Pattern in One Animal With Severe Hypocapnia (From Group C)

	pH	pCO_2 mm Hg	BB mEq/L	Na+ mEq/L	K+ mEq/L	Cl− mEq/L	HCO_3− mEq/L	ACID RAD CALS− mEq/L
Before Perfusion	7 46	25	44	150 0	3 7	109	17 4	27.3
End of Perfusion†	7 54	8	36	144 0	3 7	110	7 0	30 7
Changes	+ 08	−17	−8	−6 0	0	+1	−10 4	+3 4

†Gas mixture for oxygenator 100% Oxygen 0% Carbon Dioxide

If this metabolic acidosis were primarily due to an increase of acid radicals one may expect to find only minor changes in sodium and bicarbonate concentrations The presence of major changes indicates that this type of metabolic acidosis is not merely a piling up of acid radicals

Observations from Humans Similar studies were done in 16 humans undergoing open heart surgery Perfusion times varied from 8 minutes to one hour Perfusion rates were always above 1500 cc/sq m body surface area and were usually 1800 to 2000 cc/sq m (This corresponds to flow rates from 60 to 140 cc/kg/min) Hypocapnia was avoided Metabolic acidosis was evident to only a minor degree with an average drop in buffer base of 0 9 mEq/L (Seven of the 16 cases had no progression of acidosis at all during perfusion)

Table 6 Acid Base Balance in 16 Human Cases

PATIENTS STUDIED	BEFORE PERFUSION pH	BEFORE PERFUSION pCO_2 mm Hg	BEFORE PERFUSION BB mEq/L	END OF PERFUSION pH	END OF PERFUSION pCO_2 mm Hg	END OF PERFUSION BB mEq/L	CHANGES pH	CHANGES pCO_2 mm Hg	CHANGES BB mEq/L
16	7.32	42 7	43 4	7 32	39 2	42.5	0 0	−3 5	−9

SUMMARY

1 The nature of acid base derangements in the blood during total cardiac bypass have been studied by determining the plasma pH the plasma carbon dioxide tension and the whole blood buffer base It is important to have an awareness of the interrelationships between these components when attempting to understand the derangements produced during the support of an animal or human by a pump oxygenator

2 A minor degree of acidosis customarily appeared from anesthesia and thoracotomy alone

3 Progression of acidosis during total body perfusion was accentuated by perfusion rates below 40 cc/kg/min

4 Acidosis was produced or accentuated by lack of complete (or nearly complete) oxygenation of the arterial blood

5 Hypocapnia intentionally produced by hyperventilation of the lungs during thoracotomy or by use of suboptimal carbon dioxide concentrations

in the oxygenator during perfusion gave rise to an additional degree of metabolic acidosis This latter was thought to be compensatory in nature and produced to a degree by shifts of cation out of the plasma rather than solely by the accumulation of fixed acids in the blood

6 Observations in humans during total cardiac bypass with (1) arterial hemoglobin saturation above 90% (2) perfusion rates over 1800 cc /sq m body surface area and (3) freedom from hypocapnia revealed only a negligible degree of acidosis In some patients there was actually a regression of a mild state of metabolic acidosis which had been present prior to perfusion

7 Working with these various problems we have come to feel that in conduct of humans on a pump oxygenator the least disturbance to the patient will occur if (1) no attempt is made to induce hypocapnia either by hyperventilation prior to perfusion or by suboptimal concentrations of carbon dioxide in the oxygenator (2) the arterial oxygenation of hemoglobin is not allowed to fall below 90% (3) perfusion rates are at a minimum of 1800 to 2000 cc / sq m body surface

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MYOCARDIAL CONTRACTILE FORCE AS A MEASU[illegible] CARDIAC FUNCTION DURING CARDIOPULMONARY BYPASS PROCEDURES*

WILLIAM H LEE, JR, THOMAS D DARBY, JAMES D ASH[illegible] AND EDWARD F PARKER

During total cardiopulmonary bypass procedures, in the co[illegible] intracardiac lesions by open heart surgery, it is obviously im[illegible] have available an objective method of evaluating cardiac functio[illegible] methods currently in use for estimating cardiac function are [illegible] value under the artificial conditions of total cardiopulmonary b[illegible] the past ten years, drug produced changes in myocardial contrac[illegible] been directly measured in experimental animals by means of [illegible] gauge principle[1] The scope of this report concerns the applicat[illegible] strain gauge principle for measuring ventricular contractile fo[illegible] estimation of dynamic changes in cardiac function occurring duri[illegible] pulmonary bypass procedures Ventricular contractile force durin[illegible] bypass was measured by this principle at perfusion flow rates var[illegible] 20 cc /kg of body weight/min to 50 cc /kg /min , and were also [illegible] under the conditions of "azygos flow" (approximately 10 cc /kg /[illegible]

METHOD

Mongrel dogs weighing from 10 to 22 kg , and appearing grossl[illegible] in health, were anesthetized with sodium pentothal A right tho[illegible] was performed in the fourth intercostal space, and the femoral ar[illegible] vein were exposed in the groin A plastic cannula was placed [illegible] femoral artery, and threaded up into the abdominal aorta, fo[illegible] pressure determinations Plastic cannulae were threaded into the [illegible] and inferior vena cavae, through incisions in the right auricular ap[illegible] and tape ligatures were passed around these vessels adjacent [illegible] junction with the right atrium The right carotid artery was exp[illegible] the neck, and a plastic cannula placed in it for retrograde perfu[illegible] the aorta Cardiopulmonary bypass was carried out, by connect[illegible] previously placed cannulae to a DeWall type bubble oxygenator,[2] by a dual cross circulation type Sigma motor pump A Walton type strain gauge arch was sutured directly onto the right ventricul[illegible] and used to measure heart force changes throughout the exper[illegible] Abdominal aortic blood pressure was recorded from the femoral ca[illegible] using a Statham pressure transducer These measurements and [illegible] cardiograms were recorded synchronously on a four channel Sanborn[illegible] After all cannulations were completed, and the instruments were rec[illegible] satisfactorily control data was obtained for several minutes Perfusio[illegible] experiments were carried out in 5 groups "azygos flow", 20 cc /kg /[illegible] 30 cc /kg /min 40 cc /kg /min , and 50 cc /kg /min In the case [illegible] "azygos flow group the inferior vena cava and the superior vena distal to the origin of the azygos vein were ligated for 10 minutes

*From the Department of Surgery and the Department of Pharmacology [illegible] College of South Carolina Charleston South Carolina Supported in part by the Carolina Heart Association and the National Heart Institute

recordings made. This revealed the response to "azygos flow," since the only venous return to the heart during this period was via the azygos vein, excluding the coronary sinus return. After 10 minutes of occlusion, the ligatures were released, and recordings made until a stable level of contractile force was obtained (±10%) for a period of 15 minutes. In each of the other groups, after obtaining control data, the subject was placed on total cardiopulmonary bypass, maintaining perfusion levels of 20 cc., 30 cc., 40 cc., and 50 cc./kg. of body weight/min. of pump output for periods of 10 and 20 minutes. Similar recordings were obtained on 6 patients undergoing total cardiopulmonary bypass at known perfusion flow rates during intracardiac surgical procedures.

RESULTS

The average changes in ventricular contractile force are summarized in Figure 1. In general, these results indicate that perfusion flow rates above 30 cc./kg./min. are adequate in most cases to maintain a normal state of myocardial function as measured by cardiac contractility. In dogs placed

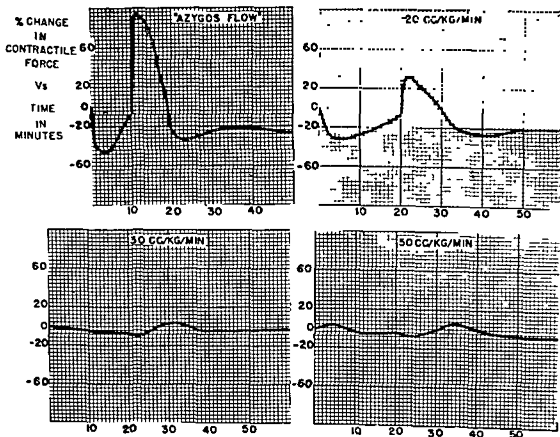


Fig 1 The percent change from the control value in heart contractile force is plotted on the ordinate, and time in minutes is plotted on the abscissa The average changes in heart contractile force produced by 10 minutes of "azygos flow" are plotted in the first graph (upper left) After a stable control period the vena cavae were occluded at 0 minutes reducing the venous return to azygos vein flow only. At 10 minutes the vena cavae were released The remaining 3 graphs illustrate the average changes in heart contractile force elicited by 20 minutes of perfusion flow rates between 20 and 50 cc/kg/min The animals were placed on total cardiopulmonary bypass at 0 minutes and removed from bypass at 20 minutes

on azygos flow,' only a marked decrease in ventricular contractile force was immediately observed This decrease in contractile force preceded changes in the ECG, which usually developed after 1 to 3 minutes of perfusion at this flow rate The ECG changes consisted primarily of a depression in the S T segment and inversion of T waves In the interval from 2 to 5 minutes, the reduction in contractile force usually reached its nadir During the remainder of the 10 minute period of reduced venous return the contractile force slowly increased Upon release of the vena cavae there occurred an immediate marked increase in contractility, usually to levels of 85 to 150% above control levels This increase lasted from 8 to 15 minutes After the period of stimulation, contractile force generally decreased to a level of approximately 35% below control and remained at this level for recording periods as long as 45 minutes At flow rates of 20 cc /kg /min , a decrease in contractile force usually occurred within the first minute ECG changes if present were again preceded by contractile force depression In several cases, the ECG displayed no abnormality In general the recordings resembled those seen during the 'azygos flow" experiments However, the changes were not as marked With flow rates of 30 to 50 cc /kg /min , only minimal changes in ventricular contractile force were observed These changes are illustrated in Figures 1 and 2 At flow rates in the range of 35 cc /kg /min or higher, no significant additional increments in contractile force were noted to occur These

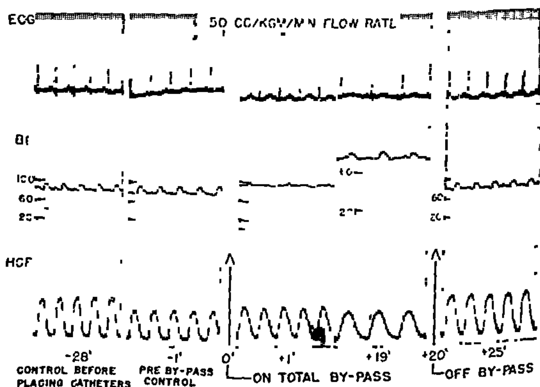


Fig 2 Illustrates the typical recordings obtained during perfusion flow rate studies at 50 cc /kg /min Electrocardiograms (ECG) abdominal aortic blood pressures (BP) and heart contractile force (HCF) were recorded Heart contractile force was directly measured by suturing a Walton Brodie strain gauge arch to the ventricular musculature of the right ventricle The heavy vertical lines represent 0 2 second time intervals

observations were confirmed in patients by the maintenance of control levels of directly measured contractile force at flow rates in the range of 35 to 50 cc /kg /min

DISCUSSION

A method of accurately determining immediate changes in cardiac function during bypass is necessary for the determination of the level of reduced perfusion flow which will best maintain the subject without introducing serious additional complications due to the adverse effects of too low or too high rates of perfusion Andreason and Watson[3] have indicated that flow rates less than 10 to 12 cc /kg /min are insufficient to maintain the vital circulation In the present laboratory studies, at flow rates in the range of 10 to 20 cc /kg /min a "rebound" increase in myocardial contractility was frequently observed to follow the initial decrease This "rebound was shown to be due to an over compensatory increase in plasma concentrations of catechol amines (epinephrine and norepinphrine) as measured by the Richardson modification of the Weil Malherbe and Bone method[4] Measured elevations in plasma catechol amines to levels of 25 to 35 times the control values were obtained during this 'rebound phase" Spontaneous ventricular fibrillation ocurred in one case displaying a hun dred fold increase in catechol amine levels during the "rebound phase"[5] Longmire *et al*[6] have presented evidence that high flow rates (in the range of 70 cc /kg /min) in the use of the bubble oxygenator, may produce changes in the blood or circulation which are permanently deleterious and apparently incompatible with survival[6] Therefore, a method of eval uating cardiac function is presented, which will with consistency reflect the adequacy of perfusion flow rates for the maintenance of vital circulation in terms of the preservation of myocardial function

The factors which influence this method and the relationships of con tractile force recordings to other methods of measuring cardiac function have been studied in detail, particularly by Cotten at the National Heart Institute[7 8 9] If the myocardium between the two sutures is stretched by 50% of its initial diastolic length, for all practical purposes the con traction of the fibers between the two points of attachment is isometric Under these conditions ventricular contractile force has been shown, in the presence of an intact circulation, to have a linear or slightly curvilinear, relationship to stroke work of the heart Measurements of the contractile force by the strain gauge arch method have been shown to be little affected by changes in other parameters of cardiovascular dynamics

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CENTRAL VENOUS PRESSURES DURING TOTAL CARDIAC BYPASS*

BERNARD S LEVOWITZ, MARIE KERNAN AND RICHARD MOYSEES

Pronounced alterations in the circulating blood volume occur rapidly in open heart surgery employing a mechanical pump oxygenator Despite the use of blood pressure, pump rate, flow rate and reservoir level as indices to regulate the blood flow, accurate replacement of measured losses has occasionally failed to re establish a normovolemic state Previous observations[1] had suggested that the central venous pressure varied directly with the blood volume and might provide a more sensitive guide to the maintenance of a normal circulating volume This study was therefore undertaken to investigate the alterations of the central venous pressure during total cardiac bypass

METHOD

Twenty three mongrel dogs weighing 13 to 23 kg were anesthetized with intravenous pentobarbital sodium (25 mg/kg) They were intubated with a cuffed endotracheal tube and ventilated by a mechanical respirator with 100% oxygen The chest was entered through the bed of the right fifth rib and the azygos vein ligated in continuity After the pericardium had been incised longitudinally the inferior and superior venae cavae were isolated extrapericardially and surrounded by sling ligatures The left subclavian artery was exposed and divided Heparin (Connaught) 25 mg/kg was administered intravenously at the completion of all dissections Plastic catheters of equal caliber (size #14F or #28F) were placed in the superior and inferior venae cavae through the right auricle and the tips

*From the Department of Surgery State University of New York College of Medicine at New York City Brooklyn and Kings County Hospital Supported by grants from the United States Public Health Service the Life Insurance Medical Research Fund American Heart Association New York Heart Association and the State University of New York

positioned distal to the sling ligatures The left subclavian artery was incannulated with size #18F catheter in all animals

The extracorporeal circuit used in these experiments has been described[7] previously in detail and consisted of a flowmeter, rotating screen disc blood filming oxygenator, bubble trap and a pair of modified Dale Schuster pumps These pumps delivered a variable minute output dependent upon the volume of venous blood present in the oxygenator reservoir Venous drainage was assisted by a fixed siphon effect equal to the vertical distance between the right atrium and the openings of the filming jets, this pressure head measured 40 cm Flows averaged 12 ml /kg /min Plastic cannulae 1 5 mm in internal diameter were threaded into the superior and inferior venae cavae via the external jugular and femoral veins, they were positioned 2 cm distal to the tips of the drainage catheters Caval and femoral artery pressures were monitored by Statham strain gauges recording on a Sanborn Poly Viso Base line readings were obtained at the level of the right auricle Flow rate and pump rate were similarly recorded

The animal was connected to the fully primed extracorporeal circuit and the heart and lungs totally bypassed by tightening the sling ligatures about the cavae At the same time the animal was taken off the mechanical ventilator Control observations were made and the circulating volume in the bypass circuit subjected to 100 ml increments or decrements of blood, which were added to the oxygenator reservoir At intervals the superior and inferior caval catheters were separately occluded and pressure readings were obtained for each vessel

In 6 animals a midline laparotomy was performed in addition to a thoracotomy A plastic catheter was threaded into the portal vein through a mesenteric venous tributary and portal pressures recorded during total cardiac bypass Simultaneous observations were made of liver size and color

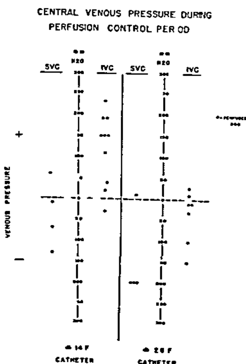


Fig 1 Venous pressure in individual dogs during control period on total perfusion showing the higher levels obtained from #14 catheter

RESULTS

The results of control readings of the central venous pressures are graphically depicted in Figure 1 Inferior vena cava pressure and flow exceeded superior vena cava pressure and flow in all perfusions Central venous pressures obtained when size #14F catheters were employed were greater than when the larger size #28F catheters were used. The latter perfusions were frequently associated with persistently negative pressure curves In the presence of a pressure head of 40 cm of H_2O, siphoning and collapse of caval walls resulted when venous pressure fell below zero mm of H_2O This was noted most often in the superior vena cava, which returned 25% to 50% of the inferior caval flow as measured by the flow meter Pressure flow curves obtained with both catheters on artificial models generally corresponded with the values seen in these experiments In dogs of approximately equal weight, higher flows were obtained when the size #28F catheter was used

Augmenting or reducing the circulating volume produced parallel alterations in caval pressures, blood pressure, flow rate and pump rate The inferior and superior cavae pressures were the most sensitive indicators of these changes Quantitative pressure variations were apparent if positive pressures prevailed but were absent when baseline or negative pressures were present The inferior vena cava demonstrated the most uniform pressure response, averaging 22 mm of H_2O pressure for each 100 ml increment or decrement of blood With a reduction in the circulating volume, depression of superior vena cava pressure occurred more rapidly than inferior cava pressure At extreme negative pressures (less than −200 mm of H_2O) achieved with the size #28F catheters, the addition of several increments of blood were required to elicit an elevation of pressure Regardless of catheter size, the pressure obtained by momentarily occluding the superior or inferior vena cava outflow (static pressure) varied directly with the circulating blood volume These points are illustrated in Figures 2 and 3

Portal pressure responded simultaneously to fluctuations in inferior caval pressure Sustained caval pressures in excess of 250 mm to 400 mm of H_2O were obtained by overloading the circulating volume when the size

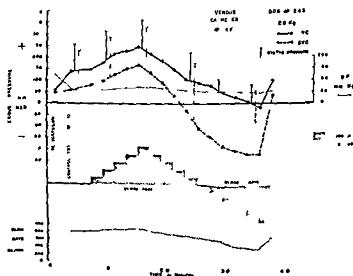


Fig 2 The effects of altering the blood volume upon the venous pressure during total perfusion when #14F catheters are used

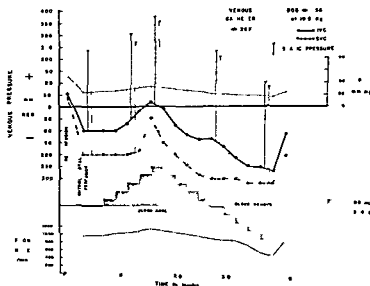


Fig 3 Blood volume and venous pressure relationships using #28F catheters Note that static pressures reflect changes in blood volume when venous pressure is negative

#14F catheter was used or by partially occluding the inferior vena cava catheter A rise in portal pressure of equal magnitude resulted together *with enlargement and congestion of the liver* The latter exhibited blunting of its edges and weeping of serous fluid from its capsule Restoration of caval pressure to running levels was followed by a slower and lesser fall in portal pressure, a decrease in circulating volume and a diminution in liver size and congestion However, cyanotic mottling frequently persisted *to the end of the perfusion period*

DISCUSSION

The behavior of the central venous pressure was similar to that found in distensible mechanical systems The influence of local or systemic autonomic and humoral factors was not apparent Venous pressure varied inversely with the outflow resistance as determined by the caliber of the draining catheters and directly as the blood flow At negative pressures the collapsible venous system was able to accommodate an increase or decrease in blood volume without a commensurate rise or fall in pressure In the distended state, i e positive pressure range, the response was generally quantitative for each increment or decrement of blood This was seen most often in the inferior vena cava where higher pressure and flows prevailed Under the variable conditions of this experiment, the circulating blood volume was best correlated with the static pressure secured by momentarily clamping the venous outflow These studies indicate that a knowledge of the baseline and running inferior vena cava phasic and static pressures will assist in the control of the circulating blood volume

Although these perfusions were conducted with inferior and superior caval pressures at positive or negative levels, no detectable difference in the rate or condition of the surviving animals was observed provided the arterial input was adequate

Sustained high positive pressures in the inferior vena cava, brought about by constriction of the outflow catheters, rapidly caused splanchnic congestion This was associated with a reduction in the circulating volume ascribed to the pooling of blood within the portal system As a contributing

factor the significance of fluid exudation from the liver warrants further investigation These effects were also demonstrated when the small caliber size #14F catheter was used in the presence of high flows The above findings suggest that in extracorporeal bypass circuits with gravity drainage selection of venous catheters which offer minimum resistance to anticipated flows is desirable

SUMMARY

The changes in the superior and inferior vena caval pressures are described in 23 animals subjected to total heart lung bypass with an extra corporeal pump oxygenator Blood flow and catheter size appear to be the principal factors concerned with the regulation of these pressures The relationship of blood flow and blood volume to the phasic and static pressures is evaluated The deleterious effects of elevated inferior vena cava pressures upon the portal circulation during perfusion are discussed

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BENEFICIAL EFFECTS OF INFERIOR VENA CAVAL OCCLUSION WHEN THE THORACIC AORTA IS OCCLUDED*

WALTER G GOBBEL JR JAMES B DALTON WILLIAM L TAYLOR H WILLIAM SCOTT JR AND ROBERT I CARLSON

Cerebral hypertension rapid cardiac dilatation and spinal cord damage are frequent problems when the thoracic aorta is occluded Cardiac dilatation and ventricular fibrillation with resultant death have been reported in cases immediately following thoracic aortic occlusion in man[3] A high incidence of hindquarter paralysis has been reported in man and dogs when the thoracic aorta is occluded for periods of 30 minutes and longer[1,5] The experiments reported here were performed in order to determine whether a method could be devised for maintaining relatively normal blood pressures above the site of thoracic aortic occlusion and avoiding cardiac dilatation Subsequent experiments were carried out to study the effect of simultaneous thoracic aortic and inferior vena caval occlusion on spinal cord survival

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METHOD

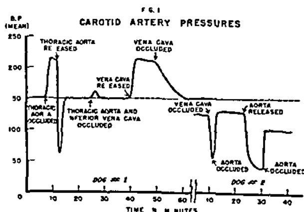
Experiment A—Measurements of Pressure and Oxygen Saturation Following Thoracic Aortic and Inferior Vena Caval Occlusion. Twenty two normothermic adult mongrel dogs were anesthetized with intravenous pentobarbital Polyethylene catheters were then introduced into the right common carotid artery, right femoral artery, superior vena cava, and inferior vena cava to record pressures An endotracheal tube was inserted and attached to a mechanical respirator The supradiaphragmatic inferior vena cava and the thoracic aorta at the level of the sixth rib were isolated through a left transpleural incision At this point baseline recordings of pulse and blood pressure were made, during which time no manipulations were carried out First, the descending thoracic aorta at the level of D6 was occluded with an aortic clamp In each animal the systolic, diastolic, and mean pressures as measured in the right common carotid artery precipitously rose (Fig 1, Dog 1) and the pressure in the right femoral artery fell The heart dilated and remaind dilated while the aorta was occluded After an observation period of 5 to 10 minutes the clamp was removed and the carotid arterial pressure fell abruptly below baseline and then returned to pre occlusion levels In other animals with only the thoracic aorta occluded the carotid hypertensive levels were maintained up to 4 hours

Next, the inferior vena cava above the diaphragm and the descending thoracic aorta were cross-clamped simultaneously (Fig 1, Dog 1) There was no significant change in systolic, diastolic, or mean pressures above the level of the occlusion and the heart did not dilate When the inferior vena caval occlusion was released while the aortic occlusion was maintained the pressures in the carotid artery again rose to levels which were attained when the descending thoracic aorta only was initially occluded The heart again immediately dilated and remained dilated during the period of thoracic aortic occlusion With re occlusion of the inferior vena cava, the carotid arterial pressure again returned to baseline levels

When the inferior vena cava alone was occluded the mean pressures in the carotid artery rapidly fell (Fig 1, Dog 2) While maintaining caval occlusion the descending thoracic aorta was clamped and the carotid arterial pressure rose promptly to baseline levels

The pressure in the inferior vena cava below the site of occlusion increased at least 100% each time the inferior vena cava was occluded

Fig 1



Oxygen saturation of the blood in the inferior vena cava fell from a mean of 50% to 3% in 35 minutes when the thoracic aorta and the inferior vena cava were simultaneously occluded The oxygen saturation in the aorta below the occlusion remained constant at 80%

Experiment B—Spinal Cord Survival Forty adult normothermic mongrel dogs were prepared as described in the experiment above with the exception that pressure measurements were not made In each case following thoracic aortic occlusion at the level of D6 5 mg of aqueous heparin was injected into the thoracic aorta immediately below the point of occlusion The dogs were divided into 2 groups Group 1 (controls) 20 dogs with thoracic aortic occlusion alone for 30 minutes and Group 2 (experimental group) 20 dogs with simultaneous thoracic aortic and inferior vena caval occlusion for 30 minutes Following termination of the occlusion the left chest wound was repaired the chest was aspirated and the dogs returned to their respective cages and observed Spinal cord survival was measured by the ability of the dog to walk within 24 hours following the procedure

Spinal cord survival was 10% in those dogs whose aortas only were occluded (Table 1) When the thoracic aorta and inferior vena cava were simultaneously occluded for 30 minutes spinal cord survival was 85%

Table 1

	THORACIC AORTA OCCLUSION ONLY	THORACIC AORTA AND INFERIOR VENA CAVAL OCCLUSION
Period of Occlusion	30 minutes	30 minutes
No of Dogs	20	20
Spinal Cord Survival	2	17
% Survival	10%	85%

DISCUSSION

Simultaneous occlusion of the inferior vena cava above the diaphragm at the time of occlusion of the thoracic aorta prevents the hypertension above the site of occlusion and the cardiac dilatation that occurs when the thoracic aorta alone is occluded Regardless of the sequence of occlusion a relatively normal carotid arterial pressure prevails if both thoracic aorta and inferior vena cava are occluded Our periods of observation following simultaneous occlusion were up to 2 hours At this time the mean carotid pressure was approximately 10 mm Hg less than the baseline pressure prior to occlusion Animals whose thoracic aortas alone were occluded maintained a hypertension above the site of occlusion for periods up to 4 hours If the sudden increase in peripheral resistance caused by occlusion of the thoracic aorta were compensated for by a significant reduction in functioning blood volume then one might expect pressures above the occluded area to remain approximately unchanged Apparently this is what occurs when the inferior vena cava is occluded Evidence for this explana

tion is suggested in the experiment where the inferior vena cava was occluded for several minutes prior to aortic occlusion thus allowing for possible pooling of a volume of blood in the lower portion of the body When the thoracic aorta is then occluded the mean pressure in the carotid artery rises but always to a level 20 to 30 mm below the previous baseline (Fig 1 Dog 2) When the reverse sequence is carried out so that the inferior vena caval occlusion follows several minutes after the aortic occlusion (Fig 1, Dog 1) there is a period of 2 to 10 minutes before the carotid arterial mean pressure returns to the baseline level In this situation apparently there is a temporary pooling of blood above the occluded thoracic aorta

The incidence of spinal cord survival in normothermic dogs following thoracic aortic occlusion increases from 10% in the controls whose aortas alone were occluded to 85% when inferior vena caval occlusion is added (Table 1) An elevated pressure and a decreased oxygen saturation in the inferior cava below the site of occlusion suggests that these are a measure of decreased flow and greater tissue absorption of the oxygen present These findings are similar to those of other investigators[2, 4] studying low blood flows with caval occlusion

SUMMARY

The simultaneous occlusion of the inferior vena cava in normothermic dogs at the level of the diaphragm affords a normotensive state above the site of thoracic aortic occlusion and increases the incidence of spinal cord survival

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AN EXPERIMENTAL STUDY INDICATING THE RELATIONSHIP BETWEEN BLOOD VOLUME AND AVAILABLE VENOUS RETURN DURING EXTRA CORPOREAL CIRCULATION*

PAUL W HERRON, JOHN E JESSEPH AND K ALVIN MERENDINO

One of the many problems which confront workers in extra corporeal circulation is the limitation of perfusion rate by an apparent decrease in venous return We have observed that there seems to be a relationship between blood volume depletion and available venous return What seemed to be relatively minor degrees of blood volume depletion have been associated with rather marked reduction in available venous return, and conversely, on occasion, during periods of poor venous return small increments of transfusion have been associated with greatly improved venous return

Donald[1] has previously reported this and has estimated that a 10% reduction in blood volume results in a 50% reduction in perfusion rate

Our purpose was to study the effects of varying blood volume on available venous return

METHOD

Adult dogs weighing between 15 and 30 kg were used in these studies Bonnycastle[2] has observed a decrease in red blood cell mass and an increase in plasma volume in dogs under pentobarbital anesthesia, and suggests that these changes are due in part to the spleen Since a tagged red blood cell method was to be used for blood volumes, splenectomy was performed on all the dogs to avoid any possible errors in interpretation due to this factor

In order to detect as accurately as possible minor variations from normal blood volume, the chromium[51] method of Donuhue and associates[3] was used for all blood volume determinations Briefly this method involves *tagging autogenous red blood cells with* Cr^{51} *by incubating* the cells with the tracer at room temperature for one hour After thorough washing with saline, a carefully weighed aliquot of the tagged cells is placed in a volumetric flask and these cells when diluted serve as a standard A larger weighed aliquot of the tagged cells is rapidly injected intravenously and venous samples are obtained at 5, 10, 15, and 20 minute intervals after the injection

The activity of each sample, expressed in counts/min /cc of blood is plotted on graph paper and the curve extrapolated to "zero time" This value is compared with the activity of the standard in the following expression

$$\text{Blood volume} = \frac{\text{wt rbc inj dog}}{\text{wt rbc in std}} \times \text{dil std} \times \frac{\text{activity of std}}{\text{activity zero time}}$$

Each animal was allowed to recover from the splenectomy, as evidenced by good appetite, adequate wound healing and stable weight Normal blood volume then was determined under anesthesia by the above method This value was used as a baseline for subsequent studies in each dog

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About one week following the control study, each animal was prepared for cardiopulmonary bypass through a right thoracotomy with a #18F catheter in each vena cava for venous return to the pump-oxygenator, and a #16F catheter in a femoral artery for perfusion of the animal. The University of Washington oxygenator[4] with Sigma motor pumps was used. Flow through the system, and therefore available venous return, could be accurately determined by carefully calibrating the arterial pump, which is arranged to pump from a reservoir and therefore against a constant resistance. By balancing the flow through the venous pump against the flow through the arterial pump, flow could be measured accurately at any given pump speed.

When no further blood loss was anticipated, the blood volume for each animal was again determined. The extracorporeal circuit was established and flow at this initial blood volume measured. Collapse of the caval wall was taken as an index of complete removal of central venous blood. The dogs were then transfused with 100 cc. increments of blood and flows were measured at each new blood volume. In this way flows at multiple blood volumes could be obtained in each animal. All operative blood volumes were compared to the control values for each individual dog, and expressed as a per cent of that value.

RESULTS

When flows, expressed as cc./kg./min. are plotted against blood volumes, expressed as a per cent of the control volume, it can be seen that there is a direct relationship between blood volume and available venous return. Figure 1 shows eight such experiments. It is apparent that although a definite relationship exists, one cannot predict flow from a known blood volume because of the wide variation between animals.

Most of the flow volume curves shown are similar in shape and have a "stair step" configuration. This similarity has led us to reason that there must be only a few factors which influence this relationship or, if multiple factors exist, they must operate in a similar direction in most dogs. It is reasonable to assume that this represents the response of the vasomotor system to changes in blood volume.

Figure 2 represents the average of these flows at each relative blood volume. The average flow at 85% of control blood volume (15% depletion) is 33 cc./kg./min. The average flow at control blood volume is 39 cc./kg./min. From this point the flow increases at a linear rate with an average flow of 68 cc./kg./min. attained at 130% of control blood volume.

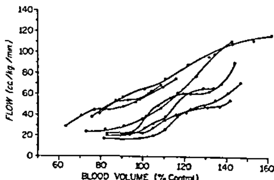


Fig. 1. Flow volume curves in 8 experiments.

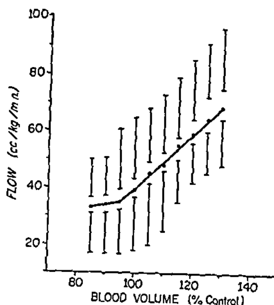


Fig 2 Average flows (dark line) at various blood volumes and ranges at each volume

Additional experiments conducted in a similar manner have shown that vasopressors increase the venous return at any given blood volume and this work will be reported in a separate publication

CONCLUSIONS

1 There is a direct relationship between blood volume and available venous return

2 In dogs the range of flows at any blood volume is too wide to allow one to predict flow from a known blood volume

3 Average flow at 85% control blood volume is 33 cc/kg/min

4 At 100% control blood volume flows average 39 cc/kg/min

5 To obtain flows approximating normal cardiac output in dogs it was necessary to expand blood volume to an average of 130% of the control

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TOTAL CARDIOPULMONARY BYPASS USING EXPERIMENTAL INTRAVENOUS OXYGENATION*

JOHN E CONNOLLY, VICTOR RICHARDS, EDMUND J HARRIS, AND SHAUN HOLMAN

Since the successful introduction of the DeWall bubble oxygenator,[1] the study of total cardiopulmonary bypass has received a tremendous stimulus In addition to the use of oxygenators in open heart surgery, significant nonsurgical applications are becoming obvious Among these possibilities is the use of the oxygenator in acute and potentially reversible cardiac or pulmonary insufficiency

The search continues for the simplest, most inexpensive, and most reliable pump oxygenator Following initial laboratory experience with the DeWall apparatus, it occurred to us that perhaps the subject might serve as his own bubbling chamber, thus allowing further simplification of what might be thought to be the ultimate in simplicity, namely the DeWall apparatus

Most investigators of the problem of intravascular oxygen administration have concluded that it is impossible to administer quantities sufficient to raise the concentration of the blood oxygen significantly without producing embolic phenomena[2, 3] Although the lungs will filter small amounts of oxygen bubbles, large amounts require "debubbling" by an artificial method

We felt that large amounts of oxygen could be bubbled into the venous system, thus raising the saturation to arterial levels, if this bubble containing venous blood was debubbled before it reached the arterial circulation Dow antifoam and a settling chamber, as employed by DeWall appeared to provide a solution to this problem Thus we elected to try bubbling oxygen into veins of all 4 extremities of the dog removing this oxygenated blood, debubbling it, and finally returning it to the arterial circulation

METHOD

Experiments were carried out on 25 adult mongrel dogs Figure 1 shows the arrangement of the apparatus Number 10F polyethylene catheters with multiple holes made with a 25 gauge needle in their terminal 4 inches were placed through a peripheral vein in each extremity and threaded into the upper and lower vena cavae near the right auricle but not in it During the bypass 100% oxygen was administered through these catheters The right chest was opened through the fourth interspace and plastic catheters approaching the size of the vena cavae were introduced into each cava the upper one through the azygos vein and the lower one through the atrial appendage Tapes were placed about the junction of the cavae and auricle for occlusion during the bypass The blood from the two cavae was then removed by gravity drainage into a burette covered by a plastic sponge soaked in Dow Antifoam This oxygenated venous blood was returned to the carotid artery of the animal via a Sigma motor pump

*From the Department of Surgery of the Stanford University School of Medicine San Francisco Aided in part by the Life Insurance Medical Research Fund

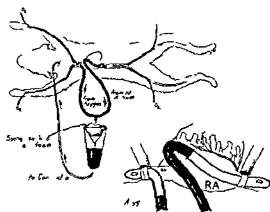


Fig 1 Diagram showing oxygen catheters in 4 extremities of dog Insert shows large catheters removing the oxygenated venous blood before it reaches the right auricle

The pump was regulated to return the amount of blood collected by gravity drainage The lungs were not inflated during the bypass Total bypass was carried out for 20 to 30 minutes

RESULTS

Of the 25 dogs only 4 survived beyond 24 hours Introduction of enough oxygen into the venous system to produce significant oxygenation of the venous blood appeared to cause a mechanical block to blood returned to the right heart via the cavae With poor return of blood to the 2 cavae we were unable to return enough oxygenated blood to the arterial circulation to maintain life in most bypassed animals Introduction of amounts of oxygen sufficient to saturate the venous blood produces increased pressure in the vena cavae and thus blocked flow into the proximal cavae and right heart

Although the production of smaller bubbles by fine holed catheters made it possible for 4 dogs to survive complete bypass for periods up to 30 minutes the great majority of the dogs succumbed with an anoxic fibrillating heart after restoration of normal circulation Oxygen saturation of venous blood removed from the right heart during bubbling ranged from 45% to 75% Higher oxygen saturation was associated with higher rates of bubbling and lower return flow and does not correlate with survival Survival appears to be related to degree of success in the removal of bubbles of oxygen before return of the blood to the arterial system We are currently attempting to produce enough small bubbles to saturate the blood without blocking flow mechanically and to better remove the microscopic bubbles before return to the arterial side This method of bypass does appear to be possible but to date it does not approach the ease afforded by the DeWall oxygenator

DISCUSSION

A safe simple pump oxygenator which could be used for periods of hours in medical emergencies such as myocardial infarction and acute right heart failure could well contribute to treatment for these diseases Present procedures for extracorporeal circulation require thoracotomy and are therefore not applicable to such medical emergencies Although partial bypass can be performed through catheters in the venous system total bypass is not possible without tourniquet of cavae To provide a simple

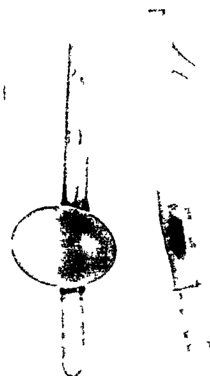


Fig 2 Photograph of ends of catheter showing holes for removal of blood proximal to balloon

method for total bypass without thoracotomy, a balloon catheter was designed by us and made by the United States Catheter Company (Fig 2) The balloon can be expanded in the proximal cavae, thus providing internal tourniquets, and the blood can be withdrawn through proximal holes These catheters can be passed through the femoral and jugular veins and guided into the proximal cavae under fluoroscopy These catheters can be modified to carry oxygen catheters In this way oxygen can be given and this oxygenated blood passed through the debubbling chamber and back to the circulation via the femoral artery (Fig 3) In this way, through 2 small incisions, complete cardiac bypass can be effected Partial bypass without oxygen could be made with the balloons not inflated This catheter can be applied to any pump-oxygenator

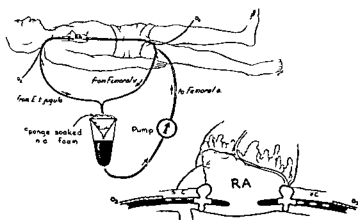


Fig 3 Possible method of total bypass in patient employing special balloon catheters

SUMMARY

1 An attempt to use the subject as his own bubbling chamber has met with only partial success The difficulties encountered are discussed

2 A catheter which can allow total cardiopulmonary bypass without thoracotomy is described

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EVIDENCE OF AIR EMBOLISM WITH THE BUBBLE OXYGENATOR COMPARISON WITH THE GIBBON OXYGENATOR*

KARL J SCHMUTZER SAMUEL A MARABLE GAUTAM DIESH
JAMES V MALONEY, JR AND WILLIAM P LONGMIRE JR

This study was prompted by the previously reported[1] finding of a significant mortality in experimental animals following cardiac bypass employing the bubble oxygenator That work demonstrated that high flow rates produced an increased mortality and that this mortality could be reduced by employing 2 bubble oxygenators operating in parallel Subsequent experiments have shown that these findings are unique to the bubble oxygenator The purpose of the present investigation was to determine whether these observations could be explained on the basis of air embolism occurring with the use of the bubble oxygenator

Microscopic observation of a capillary tube connected in parallel with the arterial line of the bubble oxygenator demonstrated the passage of occasional masses of globular foreign material It could not be ascertained whether these represented air or antifoam compound employed in the defoaming chamber Four separate physical and chemical methods were employed in an attempt to identify this particulate matter The present experiments deal with the use of a negative pressure tissue fixation technique originally described by Schubert[2, 3] for the demonstration of microscopic air embolism Schubert developed this method to demonstrate air embolism histologically in caisson disease By ordinary pathological methods air in tissue capillaries cannot be demonstrated Schubert's method consists of taking a biopsy of the suspected tissue or organ placing it in formalin and fixing it at a pressure of one half atmosphere for 5 days Intracapillary air bubbles subjected to such negative pressure will expand

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Supported by grant in aid No H 2812 from the United States Public Health Service and by the Valley Heart Fund

to twice their previous size and cause a rent in the capillary and surrounding tissue Fixation of the tissue in formalin allows the demonstration of these vacuoles after routine histologic sections are made

METHOD

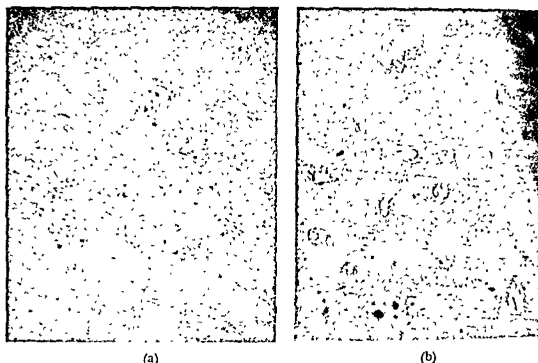
Twelve mongrel dogs weighing between 9 6 and 15 0 kg were subjected to cardiac bypass after the induction of pentobarbital anesthesia The bubble oxygenator used for these experiments was exactly the same as Model II described by DeWall and associates[4] with the following exceptions (1) in some experiments a water bath around the helical settling chamber was used to maintain blood temperature at 37°C (2) a Monel metal screen (80 mesh) was employed as a filter in the arterial line rather than the Baxter filter units described by the above authors (3) two additional bubble traps were placed in the arterial line and (4) a highly polished electroformed arterial cannula was employed instead of a plastic cannula

Before cardiac bypass a liver biopsy and nephrectomy were done Six of the animals were subjected to cardiac bypass for a period of 30 min and 4 for a period of 1 hour Nine animals were perfused at a flow rate of 70 ml /kg body weight/min and a tenth animal was perfused at 35 ml /kg body weight/min In 8 animals a 95 5% mixture of oxygen carbon dioxide was employed in the oxygenating column In 2 animals 100% oxygen was used The blood gas flow ratio in the oxygenating column varied between 1 5 and 1 10 After either 30 or 60 min of cardiac bypass a second biopsy of the liver was taken and the remaining kidney removed Immediately after discontinuation of the extracorporeal circulation the animal was sacrificed and the brain was removed The tissue specimens were placed in a desiccator containing 15% formalin and immediately subjected to one half atmospheric pressure which was maintained for a period of 5 days

Controls Each animal served as its own control since specimens were taken before and after extracorporeal circulation To increase the objectivity of the data 2 additional experiments were carried out employing a Gibbon stationary screen oxygenator The extracorporeal circuit remained exactly the same except for the fact that the stationary screen oxygenator was substituted for the bubble oxygenator Biopsies of liver kidney and brain were obtained after a 30 min period of cardiac bypass

RESULTS

Of the 10 animals examined by the negative pressure fixation technique 5 showed evidence of microscopic air embolism In 4 animals the results were equivocal and in one there was no evidence of air in the tissues Disruption of the tissues by the expanded capillary air emboli was almost invariably limited to the liver Only one brain in the series showed definite air embolism The kidneys examined were either negative or equivocal for evidence of air embolism A total of 550 microscopic slides was prepared from the biopsy specimens Mutilple sections taken from the same organ in the individual animals were remarkably consistent in demonstrating or failing to demonstrate air embolism



(a) (b)

Fig 1. (a) Control specimen of liver subjected to negative pressure fixation (b) Same animal after 20 min. bypass with bubble oxygenator. Note tissue disruption by expanded air emboli in portal spaces.

Figure 1 (a) illustrates a liver biopsy taken prior to cardiac bypass and subjected to the negative pressure fixation technique. Figure 1 (b) is a specimen of liver from the same animal taken at the end of a 30 min. period of bypass. The tissue defects resulting from the expanded capillary air bubbles in the hepatic arterial system are readily visualized. It is of interest that of the 5 animals showing bubbles in the liver, it was possible to demonstrate bubbles in only one brain and in none of the kidneys. This is not necessarily considered a sign of preferential distribution of air emboli to the liver. Rather, it is more likely the result of the fact that the parenchyma of the liver more readily demonstrates pericapillary tissue disruption under the negative pressure technique. It has been noted in brain and kidney that expanded air bubbles tend to form long clefts in the tissues following the directions of nerve fibers or renal tubules. These clefts then become very difficult to identify as definite air bubbles when the final histologic sections are made.

Controls. None of the biopsy specimens taken before cardiac bypass showed evidence of air embolism. The association between the bubble oxygenator and tissue bubbles was further strengthened by the 2 experiments in which a Gibbon stationary screen oxygenator was used. There was no evidence of air embolism either before or after cardiac bypass with the Gibbon apparatus.

DISCUSSION

These experiments do not give a definite answer to the role played by flow rates and blood – gas ratios in formation of air embolism. Previous work[1] has given inferential evidence that higher flow rates in ml./kg. body

weight/min or high total flows through the oxygenator, caused an increased experimental mortality presumably due to air embolism The data from these experiments suggest the likelihood that air embolism progressively increases with an increase in the blood—gas ratio in the oxygenating column from 1 5 up to 1 10 The demonstration of gas emboli was independent of the gas mixture (oxygen or oxygen carbon dioxide) and of the perfusion rate (35 or 70 ml/kg body weight/min) Air embolism occurred whether or not a warming bath was used around the helical settling chamber It is interesting that, despite this objective demonstration of air embolism a modicum of clinical success was achieved with this device in our early experience with pump oxygenators Our clinical experience as well as that of others suggests that man is more tolerant of air embolism than is the dog

SUMMARY

Biopsy specimens of liver kidney and brain were taken at the end of a period of cardiac bypass employing the bubble oxygenator and subjected to a special negative pressure fixation technique Five of 10 animals so examined showed gross evidence of air embolism Control specimens taken before and immediately following cardiac bypass with the Gibbon oxygenator failed to show air embolism Clinical experience with the bubble oxygenator indicates that such air embolism must be compatible with survival

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PHYSIOLOGIC CHANGES AND SURVIVAL RATE IN PROLONGED BUBBLE OXYGENATION PERFUSION WITH COMPLETE CARDIOPULMONARY BYPASS*

WILLIAM A. REED AND C. FREDERICK KITTLE

In recent years there has been much controversy regarding the feasibility and practicability of various types of oxygenators and their effects during extracorporeal perfusion. We have been particularly concerned with changes during prolonged perfusion in the bubble dispersion type oxygenator and the relation of such changes to survival. By prolonged perfusion any minor changes occurring during a short period should be intensified.

METHOD

Fifteen adult mongrel dogs weighing 12.5 to 16.2 kg. were used. Accurate body weight was obtained immediately preoperatively and postoperatively. Approximately 1200 ml. of donor arterial blood was obtained from 2 unanesthetized dogs within 30 to 45 min. of the estimated time of administration. This was collected in siliconized bottles containing heparin and normal saline † and maintained at approximately 37°F. in a water bath.

The particular type of oxygenator used has been previously described by Gott *et al.*[1] It operates on the bubble dispersion principle and is a self contained disposable plastic oxygenator.‡ The apparatus was connected with Mayon plastic tubing (¼ in. inside diameter) to latex rubber tubing (½ in. inside diameter) through Sigma motor pump heads. Stainless steel connectors with a polished internal surface were used.

The oxygenator was first primed in a retrograde fashion through the arterial outlet with normal saline that had been boiled for 10 minutes to remove dissolved gases. Meticulous and strict attention was paid to the removal of all bubbles by agitating the oxygenator and filter. Approximately 200 ml. of normal saline was then left in the oxygenator and 1100 ml. of donor heparinized blood added through the gas outlet.

After priming the oxygenator, the recipient animals were prepared for operation by the intramuscular administration of 16 mg. of morphine sulphate and 0.4 mg. of atropine. Anesthesia was induced by the intravenous administration of 2.5% solution of pentobarbital sodium maintaining the animals in as light a stage of anesthesia as possible. All animals were intubated endotracheally and ventilated with 100% oxygen with an electronically controlled ventilator cycling 14 to 16 times per minute. The animals were placed in the lateral recumbent position and right thoracotomy done. The right femoral artery was cannulated for continuous monitoring of blood pressure.

After the pleural cavity had been opened the animal was given 1.5 mg. of heparin per kg. of body weight intravenously. The azygos vein was doubly ligated and transected. A catheter was inserted in the superior vena

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†Travenol Laboratories Inc., Morton Grove, Ill.

cava through the proximal end of the azygos vein utilizing as large a catheter as possible A similar type catheter was placed into the inferior vena cava through a right atriotomy A small plastic tube with multiple holes was placed through each vena caval catheter to aid in eliminating intermittent occlusion and to improve venous outflow A plastic catheter was last inserted through the right carotid artery and passed proximally for arterial infusion

Electroencephalographic leads were inserted intramuscularly into the occipital areas and recorded by an Edin anesthograph Lead II of the electrocardiogram was also recorded by the same machine The vena caval tapes were occluded and the Sigma motor pump which had previously been calibrated was started On the arterial perfusion side the Sigma motor pump was set at a flow rate of 55 to 60 ml /kg /min After 2 to 3 minutes of operation to insure complete mixing of donor and recipient blood samples were obtained from both arterial and venous sides of the perfusion device by needling the latex rubber tubing in the pump heads The ventilator was then discontinued and the lungs allowed to collapse

A flow rate between 1 5 to 3 0 liters of O /min was used to oxygenate the blood Such a low oxygen/blood ratio is believed essential to prevent gas emboli The temperature of the disposable oxygenator and its contained blood was maintained at 36 to 38°C by means of a thermostatic heat control

The actual operation of the apparatus proved simple Occasional adjustment of venous return by varying the speed through the pump on the venous side was necessary to maintain a constant blood level in the defoaming chamber In 9 of the animals neosynephrine (approximately 300 ml of a 0 001% solution) was given intravenously to improve venous return and to maintain blood pressure In some instances it was necessary to insert a sterile polythene tubing sprayed with antifoam into the defoaming chamber after approximately 45 min of extracorporeal circulation since the usual amount of antifoam was ineffective beyond this time

Blood samples were obtained initially after 1 hour and after 2 hours of total cardiopulmonary bypass No blood was added to the extracorporeal circuit after perfusion had once been started

After 2 hours of perfusion the caval catheters were released and automatic ventilation resumed The pump was discontinued and the catheters removed Protamine was given intravenously slowly and in a single injection 2 mg /kg All animals then received approximately 400 ml of citrated fresh blood intravenously The chest was closed in anatomical layers Two animals received supplementary intravenous fluids for 2 to 4 postoperative days and all received penicillin and streptomycin daily for 7 days

RESULTS

All of the 15 animals survived the 2 hour period of total cardiopulmonary bypass One animal died 12 hours postoperatively in irreversible shock The remaining 14 animals were considered permanent survivors No late deaths have occurred Some animals have now survived 5 months and are doing well the others being sacrificed at various intervals

Table 1 Metabolic Changes Noted in Extracorporeal Circulation With Total Cardiopulmonary Bypass With Bubble Dispersion Oxygenator (Mean Flow Rate, 57 ml/kg/min)

	INITIALLY	AFTER 1 HOUR PERFUSION	AFTER 2 HOURS PERFUSION
Arterial pH	7 37	7 31	7 26
Venous pH	7 32	7 26	7 22
Arterial O_2*	18 88 vols %	18 98 vols %	18 75 vols %
Venous O_2	11 80 vols %	9 84 vols %	8 76 vols %
Arterial CO_2	26 07 vols %	23 42 vols %	21 98 vols %
	23 7 — pCO_2	23 8 — pCO	25 0 — pCO
Venous CO_2	34 89 vols %	30 13 vols %	30 39 vols %
	35 0 — pCO_2	33 8 — pCO	36 8 — pCO
Plasma Hb	41 4 mg %	87 8 mg %	128 9 mg %
W B C	10 985	9 340	8 130

*Oxygen saturation in all arterial samples was between 98 and 100%

A summary of the findings in this series of experiments is presented in Table 1 A mild metabolic acidosis was noted and was found to be progressive as noted from the pH and bicarbonate values No specific therapy for this degree of acidosis was found necessary Despite the acidosis the venous pCO_2 remained constant throughout the perfusion Plasma hemoglobin increased at the rate of 0 73 mg %/min of perfusion and did not exceed acceptable limits

It should be noted that with increasing perfusion time the venous oxygen saturation decreased

No difficulty was encountered or recognized due to gas embolization In the animal which succumbed 12 hours after perfusion examination of the brain did not disclose evidence of emboli No air bubbles were noted at any time below the first settling angle in the oxygenator Fibrin was noted in the debubbling chamber but was not observed in the angled portion or in the outflow filter

Postoperative hemorrhage occurred in one dog Following 300 ml of blood loss through the thoracostomy tube the incision was reopened and an intercostal vessel found bleeding This was ligated and the animal made an uneventful recovery Thoracentesis was done in the first 3 animals without any blood being aspirated and was subsequently abandoned as a routine measure

The blood pressure varied between 90 and 140 mm of mercury in all animals during the perfusion The average flow rate was 57 ml/kg/min Electrocardiographic tracings were normal in most animals throughout the perfusion although in a few instances there was an S T segment depression These changes returned to normal following discontinuance of the extracorporeal perfusion

ELG records revealed no significant change during perfusion in those animals surviving without neurologic damage In the animal which died there was a decrease in the frequency and amplitude of the waves These changes were also noted in some of the animals manifesting temporary neurologic changes post perfusion In 3 animals slowing to a rate of 7 to 8 waves per second with increased amplitude was noted Tracings obtained one month post perfusion in these animals were similar to those noted preoperatively and were considered normal

Seven of the 14 animals or 50% showed evidence of neurologic damage post perfusion as manifested by hemiparesis skew deviation of the eyes generalized apathy and ataxia These symptoms persisted for varying periods although all had completely recovered by 9 days post perfusion Embolization due to gas bubbles fibrin particles or the antifoam must be considered as the etiology of these post perfusion neurologic signs No gross evidence of either gas bubbles or fibrin particles was noted and it is our opinion that these changes are due to the antifoam substance A separate study is in progress to investigate this in more detail[3]

CONCLUSIONS

1 Long term survival of consecutive animals following 2 hours of extracorporeal circulation with the dispersion type bubble oxygenator and total cardiopulmonary bypass is possible One death in 15 animals occurred

2 Fourteen of 15 animals survived but 7 exhibited signs of temporary neurologic damage

3 A mild metabolic acidosis developed but this was not severe enough to require correction

4 No consistent abnormalities were noted in the electroencephalograms or electrocardiograms taken during perfusion

5 Further study is being directed toward the etiology of post perfusion neurologic changes

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THE EFFECT OF A NONOXYGENATED CORONAROPULMONARY FLOW IN CERTAIN PHASES OF CARDIAC BYPASS*

Mariano López Belio Hung H Su and Ormand C Julian

The safety of cardiac procedures under bypass conditions using a pump oxygenator depends upon meticulous attention to every mechanical physiologic and anatomic detail Discrepancies in flow resulting from the presence of a patent ductus arteriosus, an aortic insufficiency, an aorto-pulmonary fistula, over development of bronchial collateral, or some unusual increase in peripheral resistance occurring during perfusion are upsetting to the balance of flow and must be compensated

A phenomenon of similar importance, developing under certain perfusion conditions, may be observed and lends itself to simple explanation and correction This phenomenon occurs when the beginning of perfusion is accompanied by immediate cessation of lung insufflation and neither ventriculotomy nor pulmonary artery clamping is done for a period of several minutes The color of the blood in the coronary arteries becomes progressively cyanotic despite continuous delivery of bright red blood from the pump The cyanosis is not noticeably effected by increasing the amount of blood delivered into the aorta from the pump, but disappears if respiration of oxygen mixture is resumed for 2 to 3 minutes

The explanation is that during complete cardiac bypass perfusion in an anatomically normal and unopened beating heart, the venous blood from *the coronary system is returned mainly to the right side* of the heart This blood is oxygenated during circulation through the lungs only if a respirator is being used It returns to the left heart and is ejected into the ascending aorta This internal flow may be described as the coronaropulmonary flow on the basis that it is derived from and circulates through both the coronary and pulmonary systems The blood of this flow is arterial when pulmonary function is maintained and venous in nature when the lungs are not working The coronaropulmonary flow ejected from the left ventricle meets in the ascending aorta the perfusion flow which consists of oxygenated blood delivered from the pump The depth of cyanosis observed in the coronary arteries under these circumstances without lung inflation prompted these studies which demonstrate the existence and the surgical importance of the coronaropulmonary flow during the course of total cardiac bypass perfusion with an unopened beating heart

METHOD

Mongrel dogs weighing 12 5 to 31 2 kg were anesthetized with nembutal (25 mg /kg) intraperitoneally The trachea was intubated and respiration maintained with a mechanical respirator A transternal bilateral thoracotomy was done The pump oxygenator was connected to the inflow and outflow of the heart in a routine manner The venous blood drained to a bubble type oxygenator[1] and was delivered into the aorta at the rate of 40 to 55 ml /kg /min During the perfusion the pressure in the abdominal aorta was maintained between 60 and 100 mm Hg

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Supported in part by research grant (No H 2480) U S Public Health Service

RESULTS

1. Observations With Respirator On and Off. (a) Continuous observations for 1 hour were carried out in 6 dogs, 3 dogs with the respirator on, and 3 with the respirator off. No noticeable cyanosis of the coronary arterial blood developed in the group of dogs observed with the respirator on. In dogs with the respirator off, the blood in the coronary arteries became deeply cyanotic at the end of 10 minutes, and remained so throughout the 1 hour period. The color of the coronary arterial blood, however, turned to bright red when the respirator was turned on again and run for 5 minutes.

(b) Observations were made with the respirator on and off for short intervals. Changes in the color of the blood in the coronary arteries was observed on 10 occasions in 6 dogs. A slight to moderate degree of diffuse cyanosis was noticed in all dogs after the respirator had been off for 5 minutes. It disappeared after 5 minutes of resumed mechanical respiration with room air.

In 3 dogs the degree of cyanosis of the coronary arterial blood was graded from + to ++++ during 8 observations. None showed cyanosis after 15 minutes perfusion with the respirator on. After the respirator was turned off noticeable cyanosis appeared in the blood of the coronary arteries within 1 to 2 minutes. The cyanosis again disappeared within 1 minute after resumption of respiration in 7 instances and in 2 minutes in 1 instance. The degree of cyanosis varied in different dogs. At the end of a 5 minute period with the respirator off, cyanosis in the coronary arterial blood varied from + to ++ in dogs No. 1 and 2 and was ++++ in dog No. 3.

2. Observations During Manual Breathing With Nitrogen. When 100% nitrogen was used in the mechanical respirator, cyanosis appeared in the coronary arteries within 30 seconds on 4 and within 2 minutes on 2 occasions. In all instances, the cyanosis reached ++++ within 3 minutes. When the dogs were returned to mechanical breathing of air cyanosis disappeared in 1 minute on 4 observations, and at the end of 2 minutes on 2 observations.

3. Observations on Color Changes of Coronary Arterial Blood With Respirator Off and On With Drainage of Right Ventricle and Occlusion of Main Pulmonary Artery. (a) Drainage of the right ventricle in dogs undergoing prolonged periods of cardiac bypass without lung inflation prevented the appearance of cyanosis in 4 animals.

(b) Dogs were permitted to develop cyanosis of the myocardium with the respirator off. The pulmonary artery was then occluded and the right ventricle was drained through a small stab wound. In the first 30 seconds of drainage and occlusion, the cyanotic blood in the coronary arteries was replaced with oxygenated red blood in an irregular fashion, rapidly becoming complete.

Oxygen Saturation Determinations. The results of determination of oxygen content of blood from the coronary arteries and from the abdominal aorta at different intervals during total cardiac bypass perfusion with the respirator off and on are presented in Table 1. The oxygen saturation of blood from the abdominal aorta showed a fairly constant level during

Table 1

NO	BLOOD FROM	VOL % OF OXYGEN SATURATION WITH RESPIRATOR						
		ON	OFF			ON		
		15 MIN	1 MIN	3 MIN	5 MIN	1 MIN	3 MIN	5 MIN
1	C A	17 92	17 65		13 51	13 12	16 95	18 15
	A A	18 34	17 08		18.28	17 89		
2	C A	16 50	15 63	15 60	13.26	16 61	16 61	16 15
	A A	17 10	16 77		17 65	17 08	17 14	--
3	C A	17 39	17 09	13 96	12 20	15 39	16 98	16.24
	A A	18 13	17 33		17 61	18 02		17 08

C A = Coronary artery A A = Abdominal aorta.

the course of each perfusion with the respirator off and on The oxygen content of the coronary arterial blood started to drop at the end of the first minute without respiration and reached its lowest point at the end of 5 minutes It returned to its previous level 1 to 3 minutes after the respirator was turned on

DISCUSSION

The concept that coronaropulmonary flow results in poor coronary arterial oxygenation is important in several phases of the clinical use of the pump oxygenator 1) the period before the heart opened or the pulmonary artery was clamped 2) when the pump oxygenator was used in cases without cardiotomy

The experimental results delineating the cause of the myocardial cyanosis originally observed were (1) the isolated cyanosis of the blood of the coronary arteries during the phase of perfusion with the mechanical respirator off or during breathing of nitrogen, (2) the disappearance of the isolated cyanosis of the coronary arterial blood on restoration of mechanical respiration of air, or on removal of the blood from the right ventricle with occlusion of the main pulmonary artery, (3) remarkable lowering of the oxygen saturation of the blood of the coronary artery at the end of 5 minutes with the mechanical respirator off while the blood of the perfusion flow remained constantly saturated with oxygen, and (4) electrocardiographic tracings indicating progressive myocardial anoxia during the course of cessation of respiration

The coronaropulmonary flow is derived from and circulates through both the pulmonary and coronary systems, returns to the left ventricle, and thence is ejected into the ascending aorta In the ascending aorta, beyond the aortic valve the coronaropulmonary flow meets the perfusion flow, forming a zone of turbulence consisting of mixed blood from which the coronary arteries draw their blood supply The oxygen saturation of

the blood in the mixing zone can be affected by altering the oxygen content of the blood of either the coronaropulmonary flow or the perfusion flow This has been demonstrated by lowering the oxygen saturation of the blood of the coronaropulmonary flow by turning off the mechanical respirator or by substituting nitrogen during the course of total cardiac bypass perfusion

The major part of the coronaropulmonary flow is collected from the venous side of the coronary system and a smaller part from the bronchial vessels [3,4,5] draining into the pulmonary veins

The coronary flow normally comprises 3 3 to 6 7%[6] of the total resting cardiac output During total cardiac and pulmonary bypass perfusion Helmsworth[7] found the coronary flow comprised 15% of the total perfusion flow It is known that myocardial anoxia is accompanied by a large in crease in the coronary flow This may increase 2 to 3 times within 30 to 60 seconds after cessation of respiration [8] A fall of oxygen saturation below 20% of normal oxygen capacity causes maximal dilation of coronary vessels with an increase of flow up to 5 times [9] Mariast *et al* [6] have demonstrated that the coronary sinus outflow may increase by 69% when the oxygen saturation falls to between 70 and 40% and at this level it constitutes 24 6% of the total cardiac output It is obvious that the amount of coronaropulmonary flow is porportionally increased during the phase of myocardial anoxia thereby further deepening the effect of the phenomenon

During the course of total cardiac bypass perfusion with the respirator off or the breathing of nitrogen a vicious cycle is soon established The unsaturated blood from the coronaropulmonary flow constitutes a part of the coronary arterial blood supply and causes myocardial anoxia The myocardial anoxia initiates dilatation of coronary vessels[8] and thus increases the coronary inflow and outflow which directly increase the coronaro pulmonary flow and indirectly increases the stroke volume of the left ventricle The larger stroke volume of the left ventricle increases the work the myocardium must perform and thus requires more oxygen In addition when the stroke volume increases the zone of mixing in the proximal aorta will shift distally and the ostia of the coronary arteries will receive more unsaturated blood from the coronaropulmonary flow

The foregoing experimental results may reasonably be extended to human patients during the course of total cardiac bypass perfusion Precautions should be taken concerning the cessation of manual respiration It is quite obvious that manual respiration should not be abandoned before the right side of the heart is opened and should be restored once the right side of the heart is closed at the end of the intracardiac procedure

CONCLUSIONS

(1) There is evidence to indicate the existence of a coronaropulmonary flow during total cardiac bypass perfusion with an unopened beating heart

(2) The coronary arteries draw a part if not all of their blood supply from the coronaropulmonary flow mixing with the perfusion flow in the ascending aorta during the course of total cardiac perfusion

(3) Myocardial anoxia develops soon after the cessation of pulmonary function as evidenced by progressive cyanosis and progressive lowering of

the oxygen saturation of the coronary arterial blood and electrocar graphic changes

(4) This myocardial anoxia can be prevented by maintaining the pirations or by draining the right heart

(5) The surgical importance of the coronaropulmonary flow during to cardiac bypass is discussed

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BRONCHIAL ARTERY, LEFT AURICULAR BLOOD FLOW ITS RELATION TO PULMONARY DAMAGE IN EXTRACORPOREAL CIRCULATION*

JAMES B LITTLEFIELD,† PHYLLIS R INGRAM, FRANK S BLANTON, JR J FRANCIS DAMMANN, JR AND WILLIAM H MULLER, JR

One of the serious complications in experimental and clinical extracorporeal circulation has been the development of fatal pulmonary edema and hemor rhage These findings in our laboratory were most constant when standstill exceeded 30 minutes with the pulmonary artery and aorta occluded A dilated left auricle often accompanied the pulmonary changes The con tinous unoccluded bronchial artery flow was considered as a possible cause for these changes An attempt was made to prove this relationship by

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studying the response of the pulmonary vascular bed to high and normal bronchial artery flow In evaluating this problem high bronchial artery flow animals were compared with normal controls

METHOD

Fifteen mongrel dogs were anesthetized with intravenous sodium pentobarbital and positive endotracheal pressure maintained by a mechanical respirator A right thoracotomy was employed The animals were heparinized and the vena cavae cannulated through the azygos vein and right auricular appendage The azygos vein was ligated The femoral artery and caval cannulas were connected to the Clark[1] high flow, bubble pump-oxygenator in the usual manner The aorta and pulmonary artery were clamped and cardiac standstill was induced with potassium citrate A right cardiotomy was performed The systemic circulation was maintained by the pump oxygenator employing flow rates from 54 to 85 cc./kg /min The femoral artery pulmonary artery and left auricular pressures were continuously recorded Serial lung biopsies were obtained In 3 normal dogs a high bronchial artery flow had been established by left pulmonary artery ligation several months before investigation

This method of cardiac bypass was used to isolate and study the pulmonary circulation separately from the systemic pump circulation Under these conditions the pulmonary circulation is a nonfunctioning unit except for (1) the inflowing systemic bronchial arteries which enter the pulmonary circulation through pre and postcapillary communications and (2) the open pulmonary veins draining into the flaccid left auricle The cardiac septum was intact Pulmonary venous return to the left auricle can escape only by way of the open mitral valve through the unoccluded coronary circulation (proximal to the aortic clamp) into the right auricle

In these experiments we have assumed that a pulmonary capillary pressure of 30 mm Hg is capable of producing pulmonary edema in the dog

RESULTS

During Standstill The normal dogs in standstill with the pulmonary artery clamped 30 minutes or less did not show a pulmonary artery pressure rise above 30 mm Hg The pressure of the high bronchial artery flow dogs rose to between 35 and 82 mm Hg as the femoral artery pressure fell apparently due to the high systemic bronchial artery flow entering the pulmonary vascular bed The high flow dogs showed gross and microscopic pulmonary hemorrhage and edema between 10 and 30 minutes after clamping the pulmonary artery and aorta This was not true of the normal dogs (Fig 1)

One high bronchial artery flow animal in standstill for 23 minutes with the pulmonary artery unclamped permitting backflow through the pulmonary valve did not show a pressure rise above 25 mm Hg

Decompression of the left auricle during standstill lowered the pulmonary artery pressure in both groups of dogs

The pulmonary artery and left auricular pressures with the pulmonary artery clamped were approximately equal and we assume reflected the pulmonary capillary pressure in both groups of animals.

During Cardiac Recovery The recovery period following cardiac standstill

may show an intermittent rise in pulmonary artery pressure (Fig 2) In this study the pressure reached 30 to 50 mm. Hg in the high bronchial artery flow dogs One normal animal showed a rise to 75 mm Hg

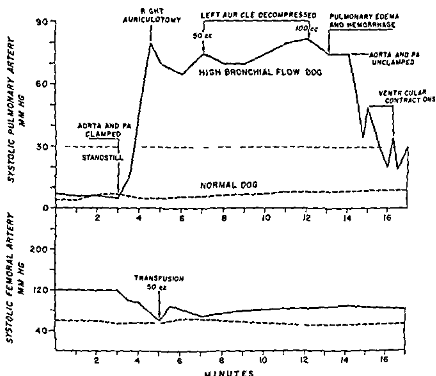


Fig 1 The pulmonary artery pressure response in a normal and high bronchial artery flow dog during total bypass with cardiac standstill

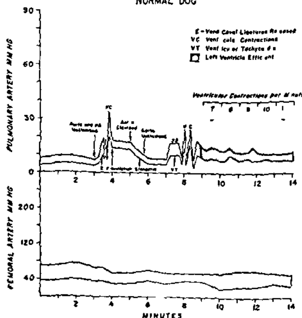


Fig 2 The pulmonary artery pressure changes during the cardiac recovery period in a normal dog

DISCUSSION

Bronchial artery flow directly influences pulmonary vascular pressure during cardiac standstill with the pulmonary artery occluded This is of more than academic interest because pulmonary capillary pressure above 30 mm Hg may produce pulmonary edema and hemorrhage In our dogs with the previously ligated left pulmonary artery, bronchial flow was high and raised the pulmonary artery pressure rapidly above 30 mm Hg within 10 to 30 minutes after standstill Pulmonary hemorrhage and edema resulted As the pulmonary artery pressure rose there was a fall in femoral artery pressure, apparently indicative of the high bronchial flow leaving the systemic and entering the pulmonary circulation These changes were not observed in our normal dogs which permitted standstill with pulmonary artery occlusion up to 60 minutes without a pulmonary artery pressure rise to dangerous levels

In both normal and high bronchial artery flow animals during standstill with pulmonary artery occlusion sufficient blood may accumulate in the lungs to elevate the pulmonary artery pressure to pulmonary edema levels Measures which may be taken to avoid these pathologic pulmonary changes are (1) an unoccluded pulmonary artery during cardiac standstill,

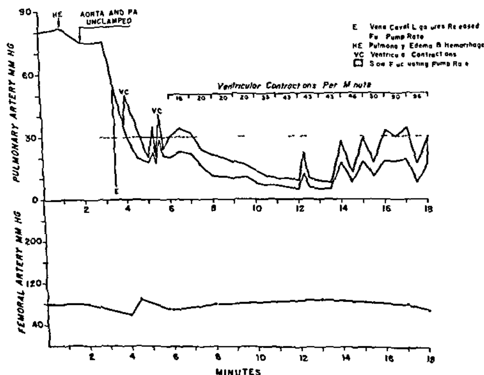


Fig 3 The pulmonary artery response of a high bronchial artery flow dog during cardiac recovery The dotted area of the graph shows the effect on the pulmonary artery pressure of a slowing pump rate before the left ventricle has completely recovered

permitting backflow through the pulmonary valve (2) The prevention of excessive systemic artery pressure in an effort to maintain a low bronchial artery flow (3) Decompression of the left auricle reducing the pulmonary vascular pressure The decompression of the dilated left auricle through the coronary circulation is inadequate in the presence of a high pulmonary venous flow

During recovery any delay in the return of left ventricular function is critical This delay may be due to fibrillation or left ventricular failure as the left ventricle attempts to overcome the aortic pressure maintained by the pump in the normal or abnormal animal Such a delay may result in a further increase in pulmonary blood volume with pulmonary hemorrhage and edema The lungs are in constant danger until the left ventricle functions efficiently (Fig 3) During recovery it seems desirable to delay closure of the right cardiotomy in an effort to keep the pulmonary artery pressure low until the left ventricle is able to overcome the pump maintained aortic pressure

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to determine the reasons for the occurrence of pulmonary hemorrhage and edema during extracorporeal circulation with cardiac standstill

The bronchial artery flow to the lungs is trapped in the pulmonary vascular bed during standstill with the pulmonary artery and aorta clamped If the bronchial artery flow is sufficiently high or the standstill prolonged pulmonary edema and hemorrhage may result

During the recovery period the efficiency of the left ventricle is important If effective right ventricular function returns first, additional blood may accumulate in the lungs raising the pulmonary artery pressure to edema levels

Preliminary studies suggest that pulmonary hemorrhage and edema may be prevented by (1) an unoccluded pulmonary artery during standstill (2) decompression of the left auricle, (3) the maintenance of a normal systemic blood pressure, and (4) delayed closure of the right cardiotomy

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THE EFFECTS OF CARDIAC BYPASS AND VENTRICULOTOMY UPON RIGHT VENTRICULAR FUNCTION*

With Report of Successful Closure of Ventricular Septal Defect By Use of Atriotomy

GEORGE R STIRLING PAUL H STANLEY AND C WALTON LILLEHEI

This study presents a simple technique for the study of right ventricular function *in vivo* and encompasses an investigation of the effects of cardio pulmonary bypass at varying rates of perfusion and of surgical incision of the ventricle upon right ventricular function The practical importance of this investigation is related to the frequency with which ventricular cardiotomy is now being used in the correction of intracardiac defects It was surprising to the authors to find several investigators have suggested that the function of the right ventricle was maintained unimpaired despite extensive damage to its myocardium by cauterization (Starr[1] Bakos[2] and Kagan[3])

Basic to this study have been the contributions of Sarnoff and Berglund[4] who have shown that ventricular performance can be clearly expressed by ventricular function curves which relate ventricular work per stroke to the mean pressure in the corresponding atrium In the experiments to be reported here the rate of blood flow into the right atrium was controlled by a pump The pressure responses in the right atrium and pulmonary artery to a series of predetermined volume loads were then studied It was assumed that the ventricular output per minute was equal to the inflow once a state of equilibrium had been reached The ventricular function curves shown here compare the right ventricular minute work with the mean right atrial pressure It is further assumed that the mean right atrial pressure bears a constant relationship to the end diastolic pressure of the right ventricle in the absence of ventriculo atrial regurgitation

METHOD

Dogs whose weights ranged from 7 kg to 20 kg were submitted to right thoracotomy under thiopentone anesthesia with constant volume positive pressure ventilation The pericardium was widely opened before the study was commenced Heparin was administered intravenously in a dose of 2 mg/kg body weight The venae cavae the azygos vein and the right common carotid artery were then cannulated and the cannulae connected to the apparatus as shown in Figure 1 Care was taken to ensure that the conduits were free of air Tapes were then passed around the caval cannulae so that when tightened all the caval blood drained by gravity into the venous reservoir V (See Fig 1)

It was then possible to either (a) return the blood thus collected to the right atrium through the arterial pumphead and the cannula in the azygos vein (Figure 1 circuit S) or (b) submit the dog to cardio-

*From The Department of Surgery University of Minnesota Medical School and The Variety Club Heart Hospital Minneapolis Supported by Minnesota Heart Association American Heart Association Graduate School University of Minnesota Life Insurance Medical Research Fund and National Heart Institute (H 830) U S P H S

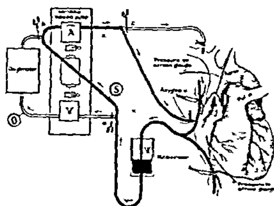


Fig 1 During a ventricular work study the caval blood is collected in the reservoir V and is returned to the right atrium via the cannula in the azygos vein The circuit is represented by the heavy black line The arterial pumphead A is calibrated before each experiment to produce a series of measured rates of flow When the clamps at a b, and c are shifted to the positions x y", and z" indicated by dotted outlines the blood courses through the venous head of the pump is oxygenated by the oxygenator and returned to the dog via the cannula in the carotid artery (stippled line) This allows a total cardiopulmonary bypass

pulmonary bypass, by leading the blood through the 'venous pumphead to a bubble oxygenator† and returning this oxygenated blood to the dog through the carotid artery cannula (Fig 1, circuit 'O) The arterial pumphead†† was calibrated before each experiment to produce a series of graded rates of flow It was found that these flows were reproducible with an error of less than 5%

Fine polyethylene tubes were then introduced into the right atrium and into the main pulmonary artery to measure the pressures The former was introduced directly while the latter was passed through a lobular branch of the pulmonary artery Full pulse curves and electronically *integrated mean pressures were continuously recorded using* Statham strain gauges and a Sanborn amplifier with a direct writing recording system

In a typical experiment the circuit 'S (Fig 1) was first used allowing the venous blood to be returned to the right atrium at a series of known rates of inflow In the experiment graphed in Figure 2 these flow rates ranged from 400 ml /min to 1500 ml /min in increments of 200 ml /min At each new flow rate 30 to 120 seconds were allowed for the pressures

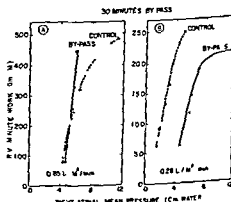


Fig 2 Comparison of right ventricular function curves obtained before and after cardiopulmonary bypass at different rates of perfusion

A Dog No 1869 8 6 kg Flow rates used in construction of the curves varied from 0 85 L/M²/min (47 ccm /kg /min) to 3.2 L/M²/min (175 ccm /kg /min) Bypass was for 30 minutes at 0 85 L/M²/min (47 ccm /kg /min) Note that ventricular function was unimpaired by bypass at this flow rate (47 ccm/kg /min)

B Dog No 98 11 4 kg Flow rates used in the work study varied from 0 8 L/M²/min (40 ccm /kg /min) to 2.2 L/M²/min (103 ccm /kg / [illegible] L/M²/ [illegible] ir [illegible] at this very low flow rate (1[illegible] ccm /kg /min)

†Disposable Bubble Oxygenator Travenol Laboratories Morton Grove Illinois The temperature of the blood was maintained at 39°C with a heater

††Sigma motor Model TM 1 Sigma Motor Inc. Middleport New York

to stabilize before the readings were taken. The body surface area was used as the basis for calculation and the flow rates ranged from 0.5 L/sq m/min to 3.2 L/sq m/min. A run at 6 graded inflow rates could be completed in 15 minutes. Care was taken to exclude the presence of ventriculo-atrial regurgitation by constant reference to a pulse recording because in the presence of regurgitation the relationship between mean atrial pressure and ventricular end diastolic pressure no longer holds.

Two such control studies of right ventricular function were made in succession, then by moving the clamps a, b and c in Figure 1 to the positions x, y and z, the dog was submitted to total cardiopulmonary bypass for a 30 minute period. The perfusion rates studied varied from 0.25 L/min/sq m surface area (16 cc/kg/min) to 1.7 L/min/sq m (90 cc/kg/min). After 30 minutes of total bypass the clamps were removed and replaced in their original positions and then 2 further series of pressure determinations were made at the same flow rates as those used in the control study.

From this data right ventricular minute work curves were plotted (Figures 2a and 2b) and the ventricular performance after bypass was compared with that in the control state.

Minute work (Gram Meters) = [Pressure in pulmonary artery (cm water) — pressure in right atrium (cm water)] × minute flow (ml) — 100 (See Fig. 2).

It should be noted that the coronary venous return to the right atrium has not been included in the minute volume nor has the acceleration component of ventricular work been included in the calculations (Sarnoff[4]).

Ten additional dogs were used to study the effect of ventriculotomy on right ventricular function. In all these animals cardiac bypass was carried out for 20 minutes at a rate of 1.2 L/min/sq m surface area (55 to 65 cc/kg/min). In 4 dogs the incision was made parallel to the interventricular groove and 1 cm from it, extending from just beneath the pulmonary valve ring to the apex of the ventricle.† In 4 other dogs the incision was made parallel to the anterior branches of the right coronary artery (perpendicular to the interventricular groove) extending from the atrioventricular ring to the interventricular groove. Two dogs were used to study the effect of a short (2 cm) incision in the outflow tract of the right ventricle (parallel to the annulus of the pulmonary valve) comparable to the incision often used for transventricular pulmonary valvotomy.

RESULTS

The normal minute work curve for the right ventricle was found to be remarkably constant (Fig. 2, control). The gradient of the curve is steep particularly at low filling pressures, indicating that a small increase in right atrial filling pressure is associated with a large increase in minute work. The lesser gradient of the curve at higher work rates is to some extent an artefact inherent in the method. The normal right ventricle accepts blood in diastole not only from the right atrium but also from the venae cavae (Rushmer[5]). In this preparation the cavae are ligated. This forces the right atrium to accept greater volumes during ventricular

†This is the incision that we have generally utilized clinically for ventricular septal defect repair.

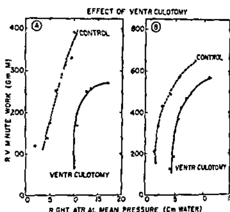


Fig 3 The impairment of right ventricular function by ventriculotomy

A Dog No 672 10 3 kg A long ventriculotomy was made parallel to the interventricular groove during cardiopulmonary bypass for 20 minutes at a rate of 1 2 L/M²/min (61 ccm/kg/min) Flows used in the ventricular work study varied between 1 1 L/M²/min (52 ccm/kg/min) and 2 7 L/M²/min (125 ccm/kg/min)

B Dog No 1 9 8 kg A long ventriculotomy was made from the atrioventricular groove to the interventricular sulcus during bypass for 20 minutes at a rate of 1 2 L/M²/min (60 ccm/kg/min) Flows used in the ventricular work study varied between 1 1 L/M²/min (55 ccm/kg/min) and 3.2 L/M²/min (164 ccm/kg/min)

systole which results in elevated mean pressures This effect is exaggerated at high inflow rates It was notable, however, that a descending limb to the curve was never observed in a normal heart—this parallels the findings of Sarnoff and Berglund[4]

When dogs were submitted to cardiac bypass at rates of flow between 0 3 and 1 7 L/min/sq m surface area for 30 minutes, the curve after bypass showed similar or improved function in every case (Fig 2A) Two dogs which were perfused at rates of flow less than 0 3 L/min/sq m surface area showed decreased ventricular function after perfusion (Fig 2B)

It was concluded from this that the cardiac bypass procedure itself within the range of flow rates that have been used in clinical practice, had no demonstrable deleterious effect on the right ventricular function of the dog

In contrast, among the dogs submitted to ventriculotomy, the minute work curve after ventriculotomy showed significant impairment of function in every case The postoperative curve (Fig 3A, Fig 3B) is displaced to the right and shows a marked flattening in the high filling pressure range In 2 cases a frank descending limb was noted indicative of ventricular failure Moreover, it was noted that in no experiment did the ventricle after ventriculotomy contribute an amount of work comparable to that seen in the control curve, towards overcoming the volume load, despite the higher filling pressure

Though the differences are slight, it seemed significant to the authors that the incision made parallel to the interventricular groove resulted in a greater impairment of function than did an incision of similar length made parallel to the branches of the right coronary artery (Fig 3A, 3B) One dog, submitted to a generous ventriculotomy, succumbed to acute cardiac failure before the studies could be completed

The 2 dogs submitted to short incisions in the outflow tract of the right ventricle showed much less disturbance of function than did the dogs submitted to longer ventriculotomies

Case Report. The deleterious effects of ventriculotomy upon postoperative function of that chamber has suggested the wisdom of closing ventricular defects in patients with severe pulmonary hypertension and increased pulmonary resistance by means of a right atrial cardiotomy The following patient had her ventricular defect successfully managed in this fashion

An 8 year old girl with congenital heart disease known since infancy was admitted to the University Heart Hospital on January 15 1957 Cardiac catheterization elsewhere in 1952 had demonstrated a ventricular septal defect with pulmonary hypertension During the last year, she had experienced a marked and progressive impairment of her already limited exercise tolerance which had concerned both her family and physician Catheterization repeated on June 25 1956 showed a right ventricular pressure of 105/5 mm Hg and a pulmonary artery pressure of 95/55 (mean 80 mm Hg) The oxygen contents of the right atrium right ventricle and main pulmonary artery were 13.5 17.2 and 16 6 volumes % respectively The systemic arterial saturation was 96% The electrocardiogram disclosed right ventricular hypertrophy with absence of the left ventricular diastolic overloading pattern

A preliminary lung biopsy and tracheotomy was carried out These lung sections showed marked medial hypertrophy in all arterioles and far advanced intimal arteriolar proliferation (Grade III lungs[6])

Corrective surgery was advised and carried out on February 15 1957 through a bilateral anterior thoracotomy incision Routine preliminary dissection of the ductus area disclosed unexpectedly, that this vessel was large and patent (1.5 cm diameter) After division of this patent ductus the thrill in the right ventricle persisted unchanged Therefore total cardiopulmonary bypass was instituted for a period of 25½ minutes utilizing the helix reservoir pump-oxygenator During this interval through a right atrial cardiotomy (without opening the ventricle) and by retracting the tricuspid leaflets a typical posterior[7] ventricular defect (1.5 cm diameter) was visualized and closed with interrupted silk sutures tied over a pledget of compressed polyvinyl sponge Potassium citrate asystole was used for 11 minutes Postoperatively respiration was aided by use of 40 to 100% oxygen administered through a respirator † By the twelfth postoperative day she was weaned completely from this unit Convalescence was uncomplicated Her systolic murmur has disappeared completely and her physical condition has improved dramatically in the 12 months since surgery

SUMMARY AND CONCLUSIONS

A practical technique for the evaluation of ventricular function has been described and used to assess the effects of an interval of cardiac bypass at varying flow rates upon ventricular function and the effects of varying types of ventriculotomies upon function

Total cardiopulmonary bypass for an interval of 30 minutes utilizing a bubble oxygenator did not show any impairment of right ventricular function at body perfusion rates from 0 3 to 1 7 L/sq m of surface area per minute Extremely low perfusion rates (below 0 3 L/sq m /min) produced definite impairment of ventricular function

A ventriculotomy always impaired ventricular function significantly The extent of this incision and its precise location were also factors in determining the degree of this impairment It is suggested that these findings have obvious clinical significance in patients with severe pulmonary hypertension A patient with severe pulmonary hypertension having a ventricular septal defect successfully closed through a right atrial rather than a ventricular cardiotomy is cited

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DIGITAL PLETHYSMOGRAPHY IN EVALUATION OF SURGERY OF DEGENERATIVE ARTERIAL DISEASE*

J LYNN EVANS E STANLEY CRAWFORD AND MICHAEL E DEBAKEY

Clinical methods are usually adequate to assess the results following operation for aneurysm and occlusive lesions of the aorta and peripheral arteries however, precise measurements of blood flow before and after operation may provide certain data that would be useful in the management of these cases The purpose of this study was to measure digital arterial inflow in a large group of patients with aneurysm and occlusive disease before and after operation The results obtained were to be correlated with the clinical assessment and since a variety of techniques were employed in these cases an excellent opportunity was available to determine and compare the effects of the various operations upon peripheral blood flow

METHOD

Mean digital arterial inflow was measured in 72 patients with aneurysm in 24 abdominal aortic occlusion in 35 and peripheral arterial occlusion in 15 patients Thromboendarterectomy was performed in 21 patients and operations requiring an arterial replacement were employed in 51 patients Lumbar sympathectomy was concomitantly performed in 36 patients 18 graft operations and 18 patients submitted to thromboendarterectomy Homografts were used in 13 and woven nylon tubes in 38 of the patients requiring an arterial replacement or bypass graft

Measurements were made in a pleasantly furnished sound proof climate control room by the plethysmographic technique described by Burch[1 2] In each patient digital blood flow was measured under conditions of vasoconstriction and maximum vasodilatation before and after operation The volume of arterial inflow was derived from the first pulse cycle using Simpson s rule and is expressed in c /mm /sec /5 cc of part

*From the Cora and Webb Mading Department of Surgery Baylor University College of Medicine and the Peripheral Vascular Laboratory of the Methodist Hospital Houston Texas Supported by Army Grant #DA 49 007 MD 630 and the Methodist Hospital Houston Texas

RESULTS

Clinically the results were essentially as expected Peripheral pulses were present before operation in the majority of patients with aneurysm and absent or reduced in patients with occlusive disease Peripheral circulation was well maintained by operation in those patients with aneurysms and peripheral pulses were restored in the majority of patients with occlusive lesions The restoration of pulses in most instances was associated with early relief of ischemic symptoms

The group mean digital arterial inflow measurements varied tremendously with the lesion and its treatment (Table 1) Digital blood flow before operation was the same in normal patients and those with aneurysms and reduced in patients with occlusions The greatest reduction was seen in the more peripheral occlusions The pattern of digital arterial blood flow remained essentially unchanged after operation in patients with aneurysms and normal patients undergoing nonvascular operations Digital blood flow in patients with occlusive lesions increased tremendously after operation and was several times as great as in normal patients and these changes were greatest in patients with occlusive lesions of the abdominal aorta That the excessive blood flow in these cases was not due to periarterial sympathectomy or reactive hyperemia is evident by the difference in blood flow at 65°F and 80°F The excessive blood flow in these cases is due to a summation of flow through the enormous collateral bed and the restored central channels

Lumbar sympathectomy was performed empirically in association with the reconstructive operation in the majority of patients with aortic occlusions because of the accessibility of the sympathetic chain at the time of aortic operation however a sympathectomy was performed in patients with peripheral occlusions or aneurysms only when peripheral blood flow was clinically severely reduced before operation or when cutaneous lesions were present The type of operation did not significantly alter the digital blood flow response in patients with aortic occlusions although vasoconstrictor tone remained in the group not sympathectomized Peripheral blood flow was slightly reduced by aneurysm replacement in patients with good pulsatile circulation before and after operation however by adding sympathectomy to the reconstructive procedure in patients with aneurysm and reduced peripheral circulation a digital blood flow response was obtained that approximated the group with good peripheral pulses A similar response was obtained in patients with peripheral occlusions Concomitant sympathectomy in patients with severe ischemic changes and secondary distal small artery occlusions produced a digital blood flow response that approximated that obtained by reconstruction operation alone in the group in which good pulses were restored

Although the mean group response was an increase in digital blood flow in all diagnostic groups the individual response varied with the type of procedure employed Digital arterial inflow was diminished after operation in 20 to 30% of the extremities in which pulses were restored by direct operative procedures alone however when lumbar sympathectomy was concomitantly performed a reduction in digital arterial inflow occurred in only 5% of the extremities involved

Table 1. Blood Flow Changes in Different Diseases (Irrespective of the Type of Operation)

DIAGNOSIS	PREOPERATIVE								POSTOPERATIVE							
	65°F				80°F				65°F				80°F			
	M	SD	N	P	M	SD	N	P	M	SD	N	P	M	SD	N	P
T	1.80	1.80	4	NS	5.40	2.27	4	NS	7.03	3.06	2	NS	11.77	3.70	2	NS
A	2.64	0.35	38	<0.001	8.33	1.23	44	<0.00	2.94	0.92	38	<0.01	8.64	1.44	44	<0.001
L	3.33	0.86	68	<0.01	8.12	1.23	71	<0.001	16.45	1.88	63	<0.001	22.08	2.20	70	<0.001
P	2.27	0.92	23	<0.05	6.13	1.61	24	<0.001	9.74	3.69	17	<0.05	14.25	2.57	21	<0.001
N	3.33	1.05	10	<0.05	8.24	3.38	11	<0.05	4.29	1.13	7	<0.01	9.35	3.68	11	<0.05

T — Thoracoabdominal aortic aneurysm
A — Abdominal aortic aneurysm
L — Partial or complete aortic occlusion
P — Peripheral segmental arterial occlusion
N — Normal limbs (control) in unilateral peripheral disease

M — Group mean of all mean digital arterial inflows at 80°F and 60% humidity (Expressed as cmm./sec./5 cc. of digit)
SD — Standard deviation
N — Number of digits studied
P — Level of significance

Table 2. Comparison of Blood Flow in Different Diseases Before and After Different Operations

DIAGNOSIS	OP	PREOPERATIVE								POSTOPERATIVE							
		65°I				80°I				65°I				80°I			
		M	SD	N	P	M	SD	N	P	M	SD	N	P	M	SD	N	P
T	D	1 80	1 80	4	NS	5 10	2 27	4	NS	7 03	3 06	2	NS	11 77	3 70	2	NS
A	D	2 73	0 37	34	<0 001	9 17	1 36	38	<0 001	2 62	0 86	31	<0 01	8 71	1 60	38	<0 001
	D+S	1 80	0 59	1	NS	3 00	1 18	6	NS	5 62	5 00	4	NS	8 20	3 70	6	NS
L	D	1 88	0 85	6	NS	8 34	1 28	9	<0 01	9 39	5 26	8	NS	21 89	6 17	9	<0 01
	D+S	3 65	0 98	59	<0 001	8 32	1 16	59	<0 001	17 64	2 05	52	<0 001	22 17	2 18	58	<0 001
P	D	4 34	1 12	13	<0 01	9 39	2 68	13	<0 01	9 59	2 51	10	<0 01	16 01	3 80	13	<0 01
	D+S	0 32	0 25	6	NS	2 32	1 12	6	NS	9 84	5 10	3	NS	11 71	3 36	6	<0 05

Diagnosis
- T — Thoracoabdominal aortic aneurysm
- A — Abdominal aortic aneurysm
- L — Complete or partial aortic occlusion
- P — Peripheral segmental arterial occlusion

Operation
- D — Direct Surgery
- D+S — Direct Surgery and lumbar sympathectomy

Keys to symbols M SD N and P as in Table 1

Table 3 Separate Record of Left to Right Toe Blood Flows in Aortic Surgery

OPERATION	NUMBER OF CASES	SIDE OF DIGIT	BLOOD FLOWS PREOPERATIVE 65°F	80°F	POSTOPERATIVE 65°F	80°F
A	23	L	3.23	9 46	3 74	9 17
		R	1 85	7 82	1 89	11 18
A S	31	L	3 05	7 53	16 99	22 05
		R	4 05	8 49	17.29	20 22

A — Aortic resection

AS — Aortic resection, endarterectomy, or bypass graft, combined with bilateral lumbar sympathectomy

Blood Flows — represent the group mean of the sum of individual mean digital arterial inflow All figures are statistically significant

The arterial inflow pattern and the character of the pulse wave was in no way associated with the type or length of the replacement employed in these cases, consequently it would appear that results in blood flow do not depend upon the physical characteristics of the replacement

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EXCISION OF THE AORTIC ARCH USING A MECHANICAL LEFT HEART BYPASS A STUDY OF THE PROBLEMS*

KEITH D J VOWLES, CECIL M COUVES AND JOHN M HOWARD

Excision of the aortic arch poses 3 main problems the maintenance of adequate myocardial oxygenation, without left ventricular overload, the provision of sufficient blood flow to the spinal cord and other distal organs, and the maintenance of adequate cerebral circulation, without hypertension Proximal to the origin of the left subclavian artery, simple crossclamping of the aorta must be supplemented by a shunt or mechanical bypass if these problems are to be overcome The use of a mechanical left heart bypass without hypothermia has proved to be experimentally adequate,[1] and the purpose of this report is to present a study of the problems concerned with this method

*From the Department of Surgery, Emory University School of Medicine Atlanta Georgia Assisted by grants from the Wellcome Trust, The Morgan Williams Bequest of the Welsh National School of Medicine and the Department of the Army U S A

METHOD

Healthy adult mongrel dogs, weighing 10 to 30 kg were anesthetized with sodium nembutal, oxygenated with a respirator and cuffed endotracheal tube, and heparinized A left thoracotomy was performed, and the aortic arch dissected out Tapes permitting occlusion were passed around the ascending aorta, the origin of the brachiocephalic artery, and the left subclavian artery Fine polythene tubes were passed into the femoral and carotid arteries and through the left subclavian artery and aortic arch to a site just above the aortic valve, for pressure measurements and collection of blood samples A J shaped stiff plastic tube 5/16 inch in diameter, containing within its lumen a fine polythene tube for ventricular pressure measurements, was inserted into the left atrium so that its opening lay just through the mitral valve The mechanical bypass system (Fig 1),

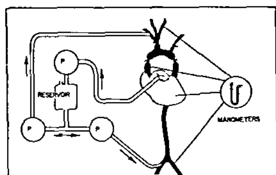


Fig 1 The left heart bypass system, showing the extraction of blood from the left ventricle and delivery to the distal systemic and cerebral circulations The points where pressure was measured are indicated

consisted of 3 variable speed finger pumps, and a one litre graduated reservoir Blood, pumped from the left ventricle to the reservoir, passed from here in 2 streams to the other 2 pumps, which returned it to the left femoral and right brachial arteries The reservoir was primed with 500 cc of heparinized blood The coronary sinus was cannulated through the right atrial appendage, an encircling suture being passed around it immediately proximal to its entrance into the right atrium Temporary tightening of this suture allowed the collection of total coronary sinus output This preparation allowed measurement of coronary sinus output, the oxygen content of coronary sinus, mixed venous and aortic blood, the blood pressure in the ascending aorta, carotid artery, femoral artery and left ventricle, and the rates of extraction of blood from the left heart and delivery to the proximal and distal segments of the systemic circulation The basic procedure was as follows control measurements were made, the pumps were then started at a relatively slow speed and the occlusive tapes tightened to isolate the aortic arch The speed of the extraction pump was then adjusted until the pressure in the ascending aorta was stable at 80 to 100 mm Hg The 2 delivery pumps were then adjusted so that the level in the reservoir remained constant, while femoral and carotid pressures were equalized Often no further adjustment of pump speeds was needed for periods of up to 1 hour

RESULTS

There was a linear relationship between the pressure in the ascending aorta and coronary sinus minute output up to about 150 mm Hg, after

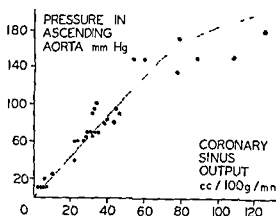


Fig 2 A graph showing the relationship between coronary sinus output (in cc /100 gm of heart weight/minute) and pressure in the ascending aorta

which an increase in pressure produced less effect on flow (Fig 2) The mean coronary sinus output before clamping the aorta, and without the bypass working, was 25 cc /100 gm of heart muscle/minute With the aorta clamped and bypass working, the pressure in the ascending aorta being 80 to 100 mm Hg, the mean coronary sinus output was 10 cc /100 gm heart muscle/minute With a pressure of 180 mm Hg the myocardium not only became suffused with blood, but in many instances tachycardia, cardiac dilatation and cardiac irregularities occurred The coronary sinus output during these high pressures reached higher values (mean 85 cc /100 gm / min) which corresponded well with the values reached when the aorta was temporarily cross clamped without the use of the left heart bypass (mean 98 cc /100 gm /min) The greatly depressed coronary flow with low aortic pressures was associated with a flabby, cyanosed myocardium, bradycardia and often ventricular fibrillation This dangerously low aortic pressure was the result of speeding up of the extraction of blood from the left ventricle until virtually no blood was passing through the aortic valve to the coronary circulation and this was a frequent cause of death in the early experiments

The mean coronary arteriovenous oxygen difference during the control period, before clamping the aorta or starting the bypass, was 15 6 vol %, compared with the mean systemic arteriovenous oxygen difference of 8 8 vol % This is the normal relationship, with the myocardium extracting much more oxygen from the blood than other tissues With the aorta clamped, and the pressure in the ascending aorta stable at 80 to 95 mm Hg and the bypass working, the mean coronary A V O_2 difference, together with the rise in coronary sinus output gave a calculated mean myocardial O_2 consumption of 1 6 cc /100 gm /min during the control period and 1 3 cc /100 gm /min when the bypass was working Myocardial oxygenation was unchanged When the extraction rate from the left ventricle was increased, reducing the pressure in the ascending aorta to 30 mm Hg or lower, the coronary sinus blood was almost totally reduced In spite of this, as a result of the very sluggish coronary flow, the mean myocardial oxygen consumption had fallen to 0 9 cc /100 gm /min The heart was blue, dilated, and grossly anoxic, for oxygen needs were far greater than supply

The total left ventricular extraction rate and cerebral and femoral delivery rates were studied when the system was balanced and stable

The left ventricular extraction rate was in the range of 20 to 50 cc/kg/min, for dogs weighing from 10 to 30 kg. A crude estimate of the total cardiac output obtained by momentarily extracting at a flow rate sufficient to produce a zero mean left ventricular pressure gave values in the range of 50 to 90 cc/kg/min. These values, which are only applicable to these highly artificial circumstances, suggest that 60 to 70% of the cardiac output was being diverted by the mechanical bypass. Usually, 60% of this was delivered to the femoral catheter for the distal circulation and 40% to the brachial for the cerebral circulation.

DISCUSSION

A left ventricular extraction rate, controlled solely by the pressure in the ascending aorta, allowed enough blood to remain in the left heart for adequate myocardial oxygenation, yet ensured the removal of enough blood to maintain the cerebral and distal systemic circulations, where the normal pressures were 40 to 60 mm Hg. There is good evidence from renal ischemia studies that these subfiltration pressures are adequate to protect at least the kidneys.[2] When higher distal systemic pressures were produced by more rapid retransfusion, venous return was augmented and blood could be extracted at an increased rate from the left ventricle, but this increased rate was never sufficient to prevent a steady drop in the level of blood in the reservoir, whereas with the pressure indicated little blood was "lost" from the system.

SUMMARY

Some of the problems encountered in the use of a partial left heart bypass procedure in exclusion of the aortic arch have been studied. Under the conditions of this experiment it was found that an ascending aortic pressure of 80 to 100 mm Hg was adequate to produce normal coronary sinus output and normal myocardial oxygen consumption. Pressures of 40 to 60 mm Hg in the femoral and carotid arteries allowed the maintenance of a stable preparation for one hour. When starting the bypass procedure it was found best to use a rate of extraction from the left ventricle of 20 to 50 cc/kg/min, 60% of this passing to the distal systemic circulation and 40% to the cerebral circulation. Once the aorta was clamped, it was imperative to base the flow rates entirely on the pressure in the ascending aorta or left ventricular cavity.

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THE EFFECT OF POROSITY IN SOLID PLASTIC ARTERY GRAFTS*

W STERLING EDWARDS

In searching for the characteristics of an ideal arterial graft, there has been much controversy about the necessity of porosity Synthetic fabrics with a moderate degree of porosity allow fibroblastic penetration through the cloth and revascularization of the new intimal lining On the other hand, porosity may cause a moderate to marked loss of blood through these pores before they become plugged with fibrin Preclotting of synthetic fabric tubes has greatly aided the insertion of these grafts and reduced loss of blood after arterial clamps are released Still, it is felt that the low incidence of thrombosis in nonporous homografts argues against the neces sity of porosity

Shumacker[9] and Self[8] studied synthetic tubes of nylon coated on the outside with an impermeable plastic, such as polyethylene or vinyl These workers found that impermeable tubes of this type had a relatively low incidence of thrombosis in arteries 1 cm in diameter, but when the caliber decreased to 6 mm in diameter the incidence of thrombosis increased considerably This is in contrast to studies with homografts[7] and permeable plastic substitutes,[3] where grafts of 6 mm internal diameter have had a low incidence of thrombosis Egdahl and Hume[4] found a much higher incidence of patency using perforated polyethylene vein lined tubes in the venous system than when they used nonperforated vein lined tubes Vas cularization of the vein graft lining of the perforated polyethylene tubes was demonstrated by India ink injection studies, whereas, no staining of veins lining solid tubes could be demonstrated Frequently maceration and discoloration of the central portion of the vein graft in the solid vein lined tubes was found Bencini and Bellinazzo[1] found the outer layer of homografts wrapped with polyethylene tubes to be necrotic and believed this demonstrated the necessity of vascularization of arterial homografts

Donovan[2] investigated the replacement of the thoracic aorta in dogs with polyethylene tubes A very small percentage of the animals main tained long term patency of these grafts Polyethylene grafts in the femoral artery were even more discouraging, all thrombosed within 2 days Lining these tubes with silicone and saturating them with heparin preoperatively did not increase the average duration of patency In 2 of the cases of thoracic aortic replacement Donovan, noted death several months after surgery from mesenteric embolism

Hufnagel[6] has used polished methyl methacrylate tubes with more en couraging results These tubes were 10 to 13 mm internal diameter and were used in the thoracic aorta of dogs Despite the report of low incidence of occlusive thrombosis in these tubes, there was an occasional late embolus to the periphery

The reported experience, therefore, with impermeable tubes has been discouraging, especially in small vessels of 6 mm internal diameter In

*From the Medical College of Alabama Birmingham Supported by U S P H Grant H 1987 (C)

With the technical assistance of Clarence Forrest and Paul Boyles

order to further study this problem and the relation of porosity to thrombosis the following studies were carried out

METHOD

Since artery grafts of 6 mm internal diameter seem to be the critical size for testing long term patency, all experiments reported here were done with grafts of this size Two types of solid plastic tubes were used, medically tested thin walled polyethylene tubes and more flexible silicone rubber tubes None of the earlier experiments using polyethylene tubes had been carried out using modern arterial suture techniques In an effort to determine the importance of porosity, approximately half of the polyethylene grafts were nonperforated, while the remaining tubes were perforated by hand using a 5 0 arterial needle point These perforations were very carefully placed about 0 25 mm apart over the entire surface of the polyethylene tube All tubes, both perforated and nonperforated, measured 2 cm in length Animals of 12 to 15 kg in weight were used and these tubes were inserted into the abdominal aorta Arterial suture anastomosis was carried out very carefully at each end to produce the smoothest possible anastomosis using 5 0 silk with an over and over stitch interrupted twice The adequacy of the perforations was easily demonstrated by the

Table 1 Nonperforated Grafts

DOG NO	GRAFT INSERTED	EXPERIMENT TERMINATED	CAUSE OF DEATH	PATENT—P CLOTTED—C
1	2/11/57	8/4/57	Sacrificed	P
2	2/25/57	3/8/57	Sacrificed	C
3	2/28/57	3/2/57	Sacrificed	C
4	2/28/57	3/2/57	Sacrificed	C
5	3/4/57	3/11/57	Sacrificed	C
6	3/4/57	8/4/57	Sacrificed	P
7	3/5/57	3/8/57	Sacrificed	C
8	3/6/57	8/4/57	Sacrificed	P
9	3/6/57	3/14/57	Sacrificed	C
10	3/6/57	3/16/57	Infection	C
11	3/20/57	3/22/57	Sacrificed	C
12	3/26/57	3/28/57	Sacrificed	C
13	3/26/57	7/10/57	Sacrificed	C
14	3/28/57	4/3/57	Sacrificed	C
15	4/16/57	5/22/57	Sacrificed	C
16	4/16/57	8/4/57	Sacrificed	P
17	4/19/57	4/29/57	Sacrificed	C
18	4/17/57	4/18/57	Sacrificed	C

fact that there was usually considerable bleeding through the openings for several minutes, and it was necessary to wrap the graft with gauze or Gelfoam until these perforations ceased bleeding. The posterior peritoneum was sutured over the graft and the abdomen closed. Femoral pulses were palpated every second day until the experiment was terminated. At the first sign of disappearance of pulses the animal was sacrificed and the graft examined.

In 10 animals silicone rubber tubes of 6 mm. internal diameter and 2 cm. length were used. These were all impermeable tubes and were sutured to the abdominal aorta in the fashion described above. These tubes were used to compare flexible tubes with rather stiff tubes, such as polyethylene.

RESULTS

As demonstrated in Table 1, there were 18 nonperforated polyethylene grafts with a long term patency of only 4 grafts (22.2%). There were 15 perforated grafts of polyethylene inserted into the abdominal aorta of dogs and long term patency of 6 (40%) (Table 2).

Of the 10 impermeable silicone tubes, all became thrombosed within one week. This is similar to the experience of Egdahl[5] using silicone rubber grafts.

Examination of the long term polyethylene grafts at the time of sacrifice from 3 to 6 months after implantation revealed some interesting findings. Of the 6 perforated grafts studied, the lining of 3 tubes was completely

Table 2. Perforated Grafts

DOG NO.	GRAFT INSERTED	EXPERIMENT TERMINATED	CAUSE OF DEATH	PATENT—P CLOTTED—C
1	2/15/57	8/4/57	Sacrificed	P
2	2/16/57	7/1/57	Sacrificed	C
3	2/19/57	3/25/57	Sacrificed	C
4	2/20/57	2/23/57	Sacrificed	C
5	2/21/57	7/10/57	Sacrificed	C
6	2/22/57	8/4/57	Sacrificed	P
7	2/22/57	2/25/57	Sacrificed	C
8	2/25/57	8/4/57	Sacrificed	P
9	2/27/57	8/4/57	Sacrificed	P
10	2/27/57	5/7/57	Infection	P
11	3/7/57	5/18/57	Unknown	P
12	3/22/57	3/29/57	Sacrificed	C
13	4/2/57	5/1/57	Sacrificed	C
14	7/9/57	7/10/57	Sacrificed	C
15	7/12/57	7/16/57	Evisceration	C

organized and pearly smooth Of the 4 nonperforated tubes, none had a completely organized lining although one tube had healing at both ends with a large area of granulation tissue in the center One of the non perforated tubes had very little organization of the lining after 6 months Characteristic of all grafts with a lining of granulation tissue, the un healed areas were much more easily detached than the completely organized surfaces It is believed that this was a critical factor in causing late throm bosis in both types of graft

DISCUSSION

There was a slightly higher patency rate in perforated than nonperforated polyethylene tubes but these groups were not large enough to make this difference significant An incidence of only 10% patency over a long period of time in tubes of this size is a very poor record in comparison with arterial homografts or permeable synthetic fabrics A possible ex planation for this may be the insecurity of attachment of the new intima of the arterial graft to the wall of the polyethylene tube regardless of perforations Homografts and synthetic grafts after several months have organized linings that are securely attached to the graft and are difficult to dissect free

It is our impression, therefore, that perforations are helpful in preventing thrombosis by allowing more rapid organization of the lining This is not the only requirement necessary to prevent the occurrence of late thrombosis The ability of a new lining to attach itself firmly to the graft is important It is believed that thrombosis after implantation of polyethylene grafts is due to partial detachment of the loose lining mem brane It is known that inflammatory reaction of degeneration in homo grafts allows the early attachment of lining thrombosis while the multiple filaments of synthetic grafts allow more secure and earlier attachment in fabric replacements

Why have fabric grafts made impermeable by an outer coat had a higher patency rate than solid plastic grafts of similar size? Perhaps this is because the lining membrane becomes more securely attached to the synthetic fibers by fibrin penetration in the former grafts The inability of fibrin clot to attach itself securely to the inside of a solid plastic may be an important consideration in constructing prosthetic valves as well as vascular grafts

The reason for the high incidence of thrombosis in the silicone rubber grafts is uncertain Silicone rubber is an inert substance and strips of this material in the subcutaneous tissue initiate very little reaction This material was used to compare flexible solid tubes with stiff solid tubes Flexibility therefore, seems not to be the critical factor in these experi ments Again the inability of fibrin to become firmly attached to the inner wall may be an important factor

CONCLUSIONS

A study of permeable and impermeable polyethylene tubes and im permeable silicone rubber tubes was carried out to determine the effect of porosity and flexibility of solid plastic tubes as artery grafts

Nonperforated polyethylene tubes had a late patency rate of 22.2%, while perforated polyethylene tubes had a long term patency rate of 40% All of the silicone rubber tubes thrombosed within one week

The slightly higher ratio of patency in the perforated tubes was not felt to be significant

It was felt that the primary cause of thrombosis in these solid grafts with a smooth inner lining was the insecure attachment of the lining membrane to the wall of the tube allowing partial detachment of this lining with occlusion of the narrow lumen

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THE USE OF EXTERNALLY SUPPORTED AORTIC HOMOGRAFTS IN THE SUPERIOR VENA CAVA*

DANIEL M ENERSON AND NICOLA GALANTE

Vascular grafts to the superior vena cava have proven much less satisfactory than similar grafts to large arteries Various types of vein substitutes have been employed experimentally but the incidence of initial thrombosis has been high[1] Furthermore homologous venous[2] and aortic[3] grafts as well as venous autografts[1] to the superior vena cava become constricted so as to completely occlude the vena cava after a month or more These complications are presumed to be due to the absence of the distending effect of a high intraluminal pressure[3] Despite these discouraging

*From the Department of Surgery Indiana University Medical Center Indianapolis and the Department of Surgery State University of New York Syracuse Aided by grants from the National Heart Council United States Public Health Service Department of Health Education and Welfare the James Whitcomb Riley Memorial Association and the Indiana Heart Foundation

experimental results clinical necessity has prompted use in patients of grafts to the superior vena cava which seemed to offer a reasonable hope of lasting function These grafts have usually consisted of segments of homologous aorta or autologous vein The clinical results have been somewhat less discouraging than the experimental reports Of the 16 cases thus far reported in the accessible literature ten may be regarded as satisfactory initial results

External supports of plastic sponge[4] have been applied to vascular grafts in the arterial system in an attempt to prevent aneurysmal dilatation but have failed to do this Egdahl Hume and Schlang[5] in 1954 reported the use of plastic tubes to support vein grafts placed into the external jugular vein and the portal vein However they employed the tubes principally to permit rapid nonsuture anastomosis

This study was undertaken to investigate factors affecting early thrombosis and later stricture the two causes of failure of grafts to the superior vena cava The primary objective was an attempt to prevent the collapse and subsequent thrombosis of aortic homografts by providing a semirigid external support of Edwards Tapp braided nylon tubing[6] A secondary objective was the determination of the effect of azygos vein patency upon the success of grafts to the superior vena cava

METHOD

Forty seven mongrel dogs 7 to 20 kg in weight were used after quarantine for at least one week prior to surgery Intravenous pentothal anesthesia was used for both induction and maintenance Under aseptic conditions using a positive pressure respirator a right fourth intercostal incision was made and the superior vena cava approached The animals were divided into 3 groups dependent upon the type of graft inserted into the cava

In Group 1 (10 dogs) the superior vena cava was grafted with braided nylon tubing A 6 cm portion of tubing was inserted side to-end into the cephalad superior vena cava and end to-side into the base of the right atrium or the intrapericardial superior vena cava A continuous 5 0 silk suture was used for each anastomosis The bypassed superior vena cava was then ligated and divided between the anastomoses In 1 animal of the 10 in this group a 4 cm segment of nylon tubing was inserted end to-end between the cut ends of the cava The azygos vein was not divided in any of the animals of this series.

Group 2 consisted of 19 animals in which an aortic homograft was inserted into the superior vena cava These grafts were preserved and sterilized with 70% ethyl alcohol for a period of 9 to 120 days and reconstituted in saline an hour before use The grafts were with 2 exceptions inserted in end to-end fashion into the divided superior vena cava The largest segment that could conveniently be inserted without angulation was used usually 3 cm in length In the first 10 animals of this group the azygos vein was not interrupted in the final 9 it was ligated and divided after the anastomoses were completed

In Group 3 (18 dogs) a homograft of aorta was externally supported with a slightly shorter segment of braided nylon tube and secured to it



Fig 1 Aortic homograft externally supported with braided nylon tubing 7 days after implantation in superior vena cava Nylon is bound in firm fibrous tissue mass that prevents collapse of aortic graft

vith a few sutures of fine silk This semirigid graft was inserted end to end nto the divided superior vena cava The azygos was ligated and divided n 12 dogs and left patent in 6

Most of the animals were sacrificed within 1 to 5 weeks in order to examine for the incidence of thrombosis Periodic cavagrams and occasional venous pressure measurements were made to check the progress of the longer term survivors

RESULTS

The 10 dogs in Group 1 in which only nylon tube was used, died or were sacrificed within 5 weeks of the operation The graft was completely occluded by thrombus in 8 dogs, partially occluded in 1 dog and patent in the one dog in which a relatively short graft was inserted end to end instead of side to end

In Group 2, in which homografts of aorta were used, most of the animals were also sacrificed early to observe thrombosis Of the 10 dogs in which the azygos was not ligated, 5 were completely thrombosed, 1 partially thrombosed and 4 were patent Of the 9 animals in which the azygos was ligated, 3 are surviving at 1 year without evidence of obstruction, the other 6 had no thrombosis when autopsied

In Group 3, in which the externally supported aortic homograft was used, there were no complete thrombotic occlusions in any of the 18 animals In 2 of the 6 dogs in which the azygos was not ligated the grafts were partially occluded Of the 12 in which the azygos was ligated,

Table 1

	TOTAL NO OF DOGS	STILL LIVING	AZYGOS VEIN	THROMBOSIS IN GRAFT		
				NONE	PARTIAL	COMPLETE
Group 1	10	0	Patent	1	1	8
Group 2	10	0	Patent	4	1	5
	9	3	Ligated	6	0	0
Group 3	6	0	Patent	4	2	0
	12	1	Ligated	9	2	0

1 animal is surviving at 1 year without evidence of obstruction and there were two partial occlusions in the remaining 11

Serial cavagrams have been made of the long term survivors but they have demonstrated no discernible difference in appearance between the supported and unsupported grafts and no progressive late narrowing of the grafts of either group has been observed The venous pressures of all the long term survivors have been within normal or slightly elevated range

DISCUSSION

The fact that there have been no complete thrombotic occlusions in the nylon supported aortic homografts to the superior vena cava suggests that external compression and collapse of other types of grafts may be an important factor in failure due to thrombosis Because the period of observation of most of the dogs was short, it cannot be judged if this externally supported graft is also less apt to show late stricture and obstruction as Gerbode[3] and others have observed to occur in the freeze dry aortic homografts

Patency or closure of the azygos vein in the superior vena caval graft preparations is an important detail that has not received emphasis in previous descriptions of experiments Ligation of the azygos vein appears to improve the results of grafts, but a patent azygos vein may produce a more sensitive experimental preparation that yields results which more nearly approximate the results of grafts in the clinical situation of chronic caval obstruction It appears from these data that external support of a graft may be of greater importance in situations in which the azygos vein is patent than in cases in which it is occluded

The high incidence of thrombosis when the nylon tube alone is used as a graft indicates that prevention of collapse of the graft does not assure freedom from thrombosis The tube, of course, has an irregular interior that probably increases thrombus formation In addition, the lumen diameter of the nylon grafts was considerably less than that of the preserved aortic segments to which the supporting nylon tube was longitudinally divided and applied The greater length of the nylon grafts also may have increased the tendency to thrombosis

CONCLUSIONS

1 Superior vena cava grafts of homologous aortic segments, externally supported with Edwards Tapp nylon tubing, thrombosed less often than unsupported grafts

2 This beneficial effect of external support of grafts to the superior vena cava was evident if the azygos vein was patent

3 Ligation of the azygos vein decreased the incidence of thrombosis in either supported or unsupported aortic segments grafted into the superior vena cava

4 Grafts of the nylon tubing alone gave the highest incidence of thrombosis

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Fig 1 Aortic homograft externally supported with braided nylon tubing 7 days after implantation in superior vena cava Nylon is bound in firm fibrous tissue mass that prevents collapse of aortic graft

with a few sutures of fine silk This semirigid graft was inserted end to end into the divided superior vena cava The azygos was ligated and divided in 12 dogs and left patent in 6.

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Patency or closure of the azygos vein in the superior vena caval graft preparations is an important detail that has not received emphasis in previous descriptions of experiments. Ligation of the azygos vein appears to improve the results of grafts, but a patent azygos vein may produce a more sensitive experimental preparation that yields results which more nearly approximate the results of grafts in the clinical situation of chronic caval obstruction. It appears from these data that external support of a graft may be of greater importance in situations in which the azygos vein is patent than in cases in which it is occluded.

The high incidence of thrombosis when the nylon tube alone is used as a graft indicates that prevention of collapse of the graft does not assure freedom from thrombosis. The tube, of course, has an irregular interior that probably increases thrombus formation. In addition, the lumen diameter of the nylon grafts was considerably less than that of the preserved aortic segments to which the supporting nylon tube was longitudinally divided and applied. The greater length of the nylon grafts also may have increased the tendency to thrombosis.

CONCLUSIONS

1. Superior vena cava grafts of homologous aortic segments, externally supported with Edwards-Tapp nylon tubing, thrombosed less often than unsupported grafts.

2. This beneficial effect of external support of grafts to the superior vena cava was evident if the azygos vein was patent.

3. Ligation of the azygos vein decreased the incidence of thrombosis in either supported or unsupported aortic segments grafted into the superior vena cava.

4. Grafts of the nylon tubing alone gave the highest incidence of thrombosis.

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	9	3	Ligated	6	0	0
Group 3	6	0	Patent	4	2	0
	12	1	Ligated	9	2	0

1 animal is surviving at 1 year without evidence of obstruction and there were two partial occlusions in the remaining 11.

Serial cavagrams have been made of the long term survivors but they have demonstrated no discernible difference in appearance between the supported and unsupported grafts and no progressive late narrowing of the grafts of either group has been observed. The venous pressures of all the long term survivors have been within normal or slightly elevated range.

DISCUSSION

The fact that there have been no complete thrombotic occlusions in the nylon-supported aortic homografts to the superior vena cava suggests that external compression and collapse of other types of grafts may be an important factor in failure due to thrombosis. Because the period of observation of most of the dogs was short, it cannot be judged if this externally-supported graft is also less apt to show late stricture and obstruction as Gerbode[3] and others have observed to occur in the freeze-dry aortic homografts.

Patency or closure of the azygos vein in the superior vena caval graft preparations is an important detail that has not received emphasis in previous descriptions of experiments. Ligation of the azygos vein appears to improve the results of grafts, but a patent azygos vein may produce a more sensitive experimental preparation that yields results which more nearly approximate the results of grafts in the clinical situation of chronic caval obstruction. It appears from these data that external support of a graft may be of greater importance in situations in which the azygos vein is patent than in cases in which it is occluded.

The high incidence of thrombosis when the nylon tube alone is used as a graft indicates that prevention of collapse of the graft does not assure freedom from thrombosis. The tube, of course, has an irregular interior that probably increases thrombus formation. In addition, the lumen diameter of the nylon grafts was considerably less than that of the preserved aortic segments to which the supporting nylon tube was longitudinally divided and applied. The greater length of the nylon grafts also may have increased the tendency to thrombosis.

CONCLUSIONS

1. Superior vena cava grafts of homologous aortic segments, externally supported with Edwards-Tapp nylon tubing, thrombosed less often than unsupported grafts.
2. This beneficial effect of external support of grafts to the superior vena cava was evident if the azygos vein was patent.
3. Ligation of the azygos vein decreased the incidence of thrombosis in either supported or unsupported aortic segments grafted into the superior vena cava.
4. Grafts of the nylon tubing alone gave the highest incidence of thrombosis.

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Fig 1 Aortic homograft externally sup with braided nylon tubing 7 days aft plantation in superior vena cava Ny bound in firm fibrous tissue mass tha vents collapse of aortic graft

with a few sutures of fine silk This semirigid graft was inserted end to into the divided superior vena cava The azygos was ligated and div in 12 dogs and left patent in 6.

Most of the animals were sacrificed within 1 to 5 weeks in ord examine for the incidence of thrombosis Periodic cavagrams and sional venous pressure measurements were made to check the progre the longer term survivors

RESULTS

The 10 dogs in Group 1 in which only nylon tube was used die were sacrificed within 5 weeks of the operation The graft was compl occluded by thrombus in 8 dogs, partially occluded in 1 dog and pa in the one dog in which a relatively short graft was inserted end to instead of side to end

In Group 2, in which homografts of aorta were used, most of animals were also sacrificed early to observe thrombosis Of the 10 in which the azygos was not ligated, 5 were completely thrombose partially thrombosed and 4 were patent Of the 9 animals in which azygos was ligated, 3 are surviving at 1 year without evidence of obstruct the other 6 had no thrombosis when autopsied

In Group 3 in which the externally supported aortic homograft used, there were no complete thrombotic occlusions in any of the animals In 2 of the 6 dogs in which the azygos was not ligated grafts were partially occluded Of the 12 in which the azygos was liga

Table 1

	TOTAL NO OF DOGS	STILL LIVING	AZYGOS VEIN	THROMBOSIS IN GRAFT: NONE	PARTIAL	COM
Group 1	10	0	Patent	1	1	8
Group 2	10	0	Patent	4	1	5
	9	3	Ligated	6	0	0
Group 3	6	0	Patent	4	2	0
	12	1	Ligated	9	2	0

1 animal is surviving at 1 year without evidence of obstruction and there were two partial occlusions in the remaining 11

Serial cavagrams have been made of the long term survivors but they have demonstrated no discernible difference in appearance between the supported and unsupported grafts and no progressive late narrowing of the grafts of either group has been observed The venous pressures of all the long term survivors have been within normal or slightly elevated range

DISCUSSION

The fact that there have been no complete thrombotic occlusions in the nylon supported aortic homografts to the superior vena cava suggests that external compression and collapse of other types of grafts may be an important factor in failure due to thrombosis Because the period of ob servation of most of the dogs was short, it cannot be judged if this exter nally supported graft is also less apt to show late stricture and obstruction as Gerbode[3] and others have observed to occur in the freeze dry aortic homografts

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CONCLUSIONS

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2 This beneficial effect of external support of grafts to the superior vena cava was evident if the azygos vein was patent

3 Ligation of the azygos vein decreased the incidence of thrombosis in either supported or unsupported aortic segments grafted into the supe rior vena cava

4 Grafts of the nylon tubing alone gave the highest incidence of thrombosis

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A SIMPLE, NONSUTURE TECHNIQUE FOR RAPID VASCULAR ANASTOMOSIS*

JORGE A RODRIGUEZ AND JESSE L WOFFORD

It is recognized that a satisfactory technique for rapid vascular anastomosis would increase the horizons of vascular surgery and organ transplantation Many investigators have used internal splints and a variety of suture techniques to expedite vascular anastomosis with varying degrees of success In devising his method for the correction of aortic insufficiency, *Hufnagel*[1] *has utilized two important principles in the production* and insertion of a plastic valve, namely, the use of a nonwettable plastic material and multiple point fixation Using these two principles that *have been proven both experimentally and clinically, a technique for* rapid vascular anastomosis has been devised and forms the basis of this report

METHOD

Acrylic tubing is fashioned into 2 couplings The couplings are composed of 2 cylinders (A and B) of slightly smaller diameter than the ends of vessels (Fig 1) They are constructed in such a manner that one fits snugly into the other The internal surface of the smaller coupling is highly polished to prevent thrombosis formation Each cylinder has an external groove where a loop of stainless steel wire has been secured The long, free ends of this loop are twisted together (C) Several knots are tied in a heavy silk suture, approximately ½ mm apart This suture with knots provides adequate multiple point fixation when placed around the vessel fixing it to the circumference of the cylinder (D) The knots in the number 4 silk suture should cover a distance which is about the same as the circumference of the groove of the cylinder This is necessary *in order to* permit accurate securing of the vessel end over the cylinder

The technique of insertion of the couplings is shown diagramatically in Figure 2 *The segment of vessel is crossclamped and the vessel is divided*

*From the Department of Surgery University of Mississippi Medical Center Jackson
Supported in part by a grant from the Mississippi Heart Association

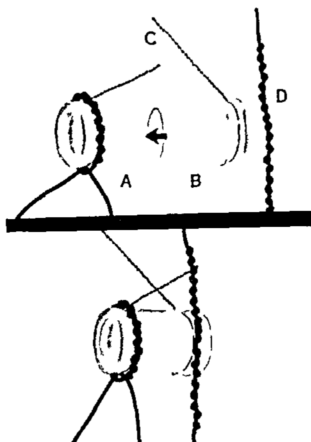


Fig 1 Photograph of anastomotic coupling device In the upper half of the photograph the two ends of the device are shown and at the lower half the coupling has been fitted together (A) is the external coupling (B) the internal coupling (C) the stainless steel safety device and (D) the knotted heavy silk suture that permits multiple point fixation

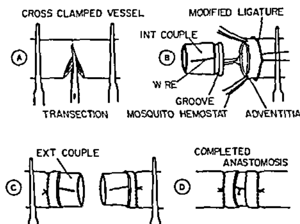


Fig 2 Diagramatic presentation of the technique for the insertion of an anastomotic coupling to be used in rapid vascular anastomosis

(A) Three small hemostats to be used for traction are placed upon the adventitia at the vessel end One half of the coupling is inserted into the lumen of the divided vessel and anchored to the vessel wall by tying the specially prepared knotted suture around the vessel at the site of the groove (E) The other, or outer, half of the coupling is inserted in the opposite vessel end in the same manner (C) The lumina of the two vessel ends are flushed with normal saline to remove any clots The anastomosis is accomplished by fitting the 2 couplings snugly together The resultant anastomosis is prevented from separation by twisting the free ends of the wires together (D) The distal clamp is removed first

RESULTS

The results of insertion of the anastomotic coupling in 23 dogs are shown in Table 1 In the early part of the study, the coupling was inserted into the descending aorta of 3 dogs The coupling became disconnected and these dogs died After this experience, a safety device was added to the coupling Stainless steel wire as shown in Figure 1 (C) was added to the device After using the wire there were no deaths in the group of dogs in which the coupling was inserted in the descending aorta The 10 dogs that survived were sacrificed at varying periods from 12 days to 3 months There was no thrombosis within the lumen of the vessel and coupling and no necrosis of the wall of the vessel There was a formation of fibrous connective tissue which covered the exposed portion of the coupling and sutures

In 10 dogs the coupling was placed in the ascending aorta under normo thermic conditions In 3 instances the animals died because of technical difficulties As more experience was gained, the coupling could be inserted much faster In 5 animals survival ranged from 14 to 36 hours but the animals eventually died of brain damage One animal survived 7 days and died of a hemorrhage because of a rupture of the ascending aorta At necropsy it could be seen that trauma to the intima of the vessel at the time of insertion led to necrosis and rupture It is aparent that the

Table 1 Results of Insertion of Anastomotic Coupling in 23 Dogs

POSITION	NUMBER OF DOGS	SURVIVAL TIME	CAUSE OF DEATH
Descending Aorta (13 Dogs)	4	Survived	Sacrificed 3 months postop
	3	Survived	Sacrificed 1 month postop
	1	Survived	Sacrificed 12 days postop
	2	Survived	Later expired 1 month postop of diarrhea
	1	7 Days	Disconnection of coupling
	2	During Operation	Disconnection of coupling
Ascending Aorta (10 Dogs)	1	7 Days	Rupture of vessel due to trauma at insertion
	2	36 Hours	Brain damage
	2	23 Hours	Brain damage
	1	14 Hours	Brain damage
	3	At Operation	Technical difficulties

results in the ascending aorta have not been as good as insertion in the descending aorta. On the other hand it is recognized that with greater experience the technical facility with which insertion can be accomplished in the ascending aorta should improve with associated improvement in survivals.

SUMMARY

A technique for vascular anastomosis that can be accomplished in approximately 2 minutes using nonwettable plastic material and multiple point fixation has been used in the ascending and descending aortas of 23 dogs. Autopsies of the survivals have not shown any significant degree of thrombosis in the couplings and no necrosis of the vascular wall.

It appears that this simple technique may have a broad application in vascular grafting, bypass procedures and in organ transplantation.

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THE IMPORTANCE OF MEASURING VENTILATION DURING THE STEADY STATE*

THOMAS F NEALON, JR, JOYCE E PRICE AND JOHN H GIBBON, JR

Carbon dioxide excretion is generally accepted as an indication of metabolic activity of the body Various drugs, anesthetic agents and planes of anes thesia have all been found to affect the output of carbon dioxide by the lungs[2 3] These reports use the terms carbon dioxide output and carbon dioxide production interchangeably and the assumption is that they are identical We propose to show that in human subjects under anesthesia, retention or depletion of carbon dioxide in the blood will result in marked variations in carbon dioxide output Under these circumstances production and output are not the same When the tension of carbon dioxide (pCO_2) of the blood is steady the output, which is then the same as to the produc tion, is more nearly constant

METHOD

Studies were carried out on patients undergoing operation under general anesthesia Anesthesia was maintained with nitrous oxide or ether and oxygen was administered by a cuffed endotracheal tube attached to a closed circuit anesthetic system Ventilation was provided by a mechanical ventilator in 19 cases and by intermittent manual compression of the anes thetic bag in one instance Ventilatory volume was measured by a gas flow meter on the expiratory arm

The concentration of carbon dioxide in the respired gases was measured by a continuous infra red absorption carbon dioxide analyzer A 3 way stopcock on the proximal end of the analyzer head made it possible to draw gas for analysis either from the end of the endotracheal tube or from the mixing chamber *The entire sampling apparatus has been de* scribed in detail elsewhere[4]

The concentrations of carbon dioxide in the inspired gas and in the end expired gas were determined at various intervals during the operation by analysis of the gas drawn from the end of the endotracheal tube The latter of these concentrations was used as a monitor of the adequacy and the constancy of ventilation The concentration of carbon dioxide in the mixed gas was used to calculate the carbon dioxide output/sq m of body surface area by the formula

*From The Department of Surgery Jefferson Medical College Philadelphia Supported in part by a grant from the U S Public Health Service

$$CO_2 \text{ output} = \frac{(F_E CO_2 - F_I CO_2 \; (V) \; (f)}{B \; S \; A}$$

when $F_E CO_2$ is the concentration of CO_2 in the mixed expired gas
$F_I CO$ is the concentration of CO_2 in the inspired gas
V is the ventilation in L/min
f is the correction factor to convert BTPS
(body temperature and pressure saturated with water vapor) to
STPD (0°, 760 mm Hg, dry)
B S A is body surface area in M^2

A variation of less than 0 1% in the concentration of carbon dioxide in the end expired gas over a period of at least 6 minutes was chosen as a practical criterion for the existence of a "steady state," i e, equilibrium between carbon dioxide production and output This represents a change in the pCO_2 of less than 1 mm Hg

Table 1 Carbon Dioxide Output Determination ml/min/M²

CASE	OPERATION	MEASUREMENTS TIME SPAN		STEADY STATE		ALL MEASUREMENTS	
		HRS	MIN	NUMBER	RANGE	NUMBER	RANGE
1	Esophagectomy	7	20	14	84 121	27	68 121
2	Resection of aortic aneurysm (hypothermia)	4	15	4	76 95	12	64 101
3	Gastrectomy	2	15	5	123 136	11	109 203
4	Gastrectomy	1	20	4	87 102	6	87 105
5	Colectomy	3	10	4	81 91	8	74 97
6	Mitralotomy	2	3	4	84 96	7	84 169
7	Excision rectum	2	0	4	126 145	6	126 160
8	Esophagectomy	1	5	5	88 113	8	88 184
9	Gastrectomy	1	10	3	99 126	5	99 154
10	Repair of hiatal hernia	2	10	2	96 101	4	96 121
11	Mastectomy		20	1	98	2	98
12	Thoracotomy	1	25	1	135	4	75 157
13	Lobectomy	3	10	1	85	5	82 122
14	Mastectomy		30	1	102	3	102 128
15	Resection of aortic aneurysm	3	20	1	115	5	75 136
16	Excision tumor parotid		40	1	96	4	88 105
17	Spleno renal shunt (manual)	7		8	97 134	18	94 184
18	Cholecystectomy		35	2	131 146	5	89 150
19	Gastrectomy	2		3	119 132	10	79 161
20	Colectomy	1	45	3	107 122	8	57 157

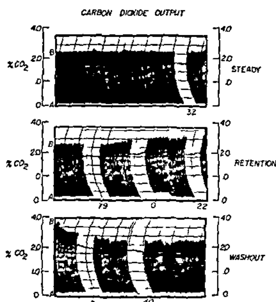


Fig 1 Tracing of the concentration of carbon dioxide in the respired gases during portions of the operation on case 19 Each vertical line of the graph represents 1 minute *B represents the concentration of carbon dioxide in the end expired gas* and A the concentration in the inspired gas The smooth segment of the tracing above the numerals indicates the concentration in the *mixed expired gas The numerals list the* carbon dioxide output at that point In the upper tracing the concentration in the end expired gas remained unchanged for 13 minutes T[illegible] *bumping t* [illegible] ing illustr [illegible] The sharp change of A in the middle of this tracing is due to a canister change The lower tracing illustrates washout of CO_2 *(B dropping)*

RESULTS

A steady state (as defined above) was realized in 20 patients (Table 1) These represent 3 different categories 16 cases (#1 16) ventilated with a mechanical respirator in which a normal state was attempted 1 case (#17) ventilated by intermittent manual compression of the bag again aimed at a normal state and 3 cases (#18 #19 #20) ventilated with a mechanical respirator in which the volume of ventilation was altered in order to change carbon dioxide output Determinations of carbon dioxide output during several steady periods were obtained in each of 14 cases in 10 of these the range of values was less than 20 ml per minute On the other *hand in 9* of these 14 patients the range CO_2 output was more than 40 ml in 6 cases more than 80 ml during changing states

In all cases studied the output during the steady state was between 76 and 146 ml/min M^2 A part of the tracing from which the figures *in case 19 were derived is reproduced in Figure 1 This illustrates* the determination of carbon dioxide output under steady conditions and the changes measured during washout and accumulation of carbon dioxide These changes gradually level out and the output approaches that of the steady state as equilibrium is reached

DISCUSSION

The tension of carbon dioxide (pCO_2) in the arterial blood of a normal individual approximates 40 mm Hg In conscious patients the tension of carbon dioxide in the end expired gas is nearly identical to that of the arterial blood [1] Under anesthesia the discrepancy between the two is greater but it is constant for any one individual and changes in carbon dioxide concentration in one are directly reflected in the other [4] Consequently an unchanged level of carbon dioxide in the end expired gas is a satisfactory indication of a constant tension of carbon dioxide in the arterial blood If more carbon dioxide is produced body mechanisms are stimulated to increase ventilation to maintain the normal level However hyperventilation will depress the arterial pCO_2 and hypoventilation will cause it to

rise Under anesthesia the regulating mechanisms are less sensitive and the ventilation is more likely to differ from that required to maintain a normal tension of carbon dioxide in the blood In the first 16 cases, in which a mechanical ventilator capable of delivering constant volumes without change for long periods was used, a steady state was infrequently achieved Fluctuations were even more noticeable in the one case of manually assisted ventilation

In the majority of cases a steady state was accompanied by a fairly small variation in output of carbon dioxide On the other hand when the state was changing the range of values was wide (up to 100 ml) Observations on these cases were not necessarily made at highest or lowest outputs Since the changing state is related to changes in the content of the blood, only when the pCO_2 of the blood remains the same is one justified in assuming that the output of carbon dioxide represents all the carbon dioxide produced

The establishment of a steady state is likewise required in determining the relationship between ventilatory volumes and the pCO_2 of the blood When the ventilation is increased the pCO_2 initially drops and then levels off (Fig 1, B, during washout) Therefore, the pCO_2 must be constant before a specific pCO_2 can be considered the result of a given volume of ventilation

SUMMARY

Output of carbon dioxide was measured at various times in 20 patients undergoing surgical procedures under general anesthesia Individual out puts varied widely during the course of operation On the other hand, when the pCO_2 of the blood was maintained constant, there was little variation in CO_2 output

The importance of determining that the pCO_2 of blood is unchanged, in evaluating the ventilatory state of the patient, is stressed

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INFLUENCE OF POSITIONAL CHANGE ON BLOOD FLOW TO SEPARATE LUNGS *IN VIVO* STUDIES*

ALBERT MOWLEM AND GILBERT S CAMPBELL

It has long been of interest from a clinical as well as a physiologic stand point to determine the effect of changes in body position on the blood flow and ventilation of each lung Early[1 2 3] and more recent investigators[4] have demonstrated from differential bronchospirometric studies increase in oxygen consumption and ventilation in a lung when it is placed down wards in the lateral decubitus position compared with observations obtained when the same lung is in the superior position The authors of these studies interpreted these findings by suggesting that blood flow through the lung increases when it is placed in a dependent position Their conclusions were based mainly upon the indirect evidence of increase in oxygen consumption The mechanism for this increase in flow was sug gested[2] to be increase in the pulmonary artery pressure and decrease in vascular resistance[3] when the lung is dependent, although no critical measurements were obtained to calculate these values

The present study was, therefore, undertaken in dogs in which actual blood flow through each lung was calculated while simultaneously recording pressures in the corresponding pulmonary artery and vein (inferior pul monary vein used), thus enabling calculation of the vascular resistance It was, therefore, posible to obtain values for blood flow, vascular resistance, and pulmonary vascular pressures on each lung in each lateral decubitus position

METHOD

Twenty mongrel dogs were anesthetized with sodium pentothal (35 mg/kg) A tracheotomy was performed and a specially made double lumen polyethylene tube was inserted into the trachea Through a left thora cotomy incision the position of this tube was checked, and it was secured with ligatures in such a manner as to isolate completely one lung from the other Care was taken not to allow the longer portion of the double lumen tube introduced into the left main bronchus of the dog to obstruct the left upper lobe bronchus which in this animal has a high take-off (usually about 1 cm distal to the bifurcation of the trachea) With the chest still open, small polyethylene catheters suitable for obtaining blood samples and measuring pressures were introduced into the right and left pulmonary arteries and the right and left inferior pulmonary veins (through the right ventricular outflow tract and the left atrium respectively) and were exteriorized near the midline of the anterior chest wall The left chest was then closed, a catheter being left in the pleural cavity and connected to suction maintained at about 15 cm of water, and the dogs were allowed to breathe on their own

Following an interval of about ½ hour to 1 hour, with the dogs still under light anesthesia, each lung was connected to a separate Benedict Roth closed system spirometer filled with 100% oxygen In this manner

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With the technical assistance of Mrs Rose Weiner

oxygen uptake by each lung was measured simultaneously over a 10 minute period with the dogs first placed in the left lateral decubitus position and later turned to the right lateral decubitus position The narrow transverse diameter of the thorax of the dog prevented accurate determinations in the supine position Halfway during recordings of oxygen consumption mean integrated pressure measurements were obtained at the levels of the tips of the catheters using Statham strain gauges and blood samples were drawn anaerobically from the catheters in the pulmonary arteries and veins for manometric determination of the oxygen content It was thus possible to calculate the blood flow to each lung by the Fick principle using the oxygen consumption and the difference in the oxygen content in the arterial and venous blood samples Vascular resistance in each lung was calculated using the formula resistance $= \frac{\Delta P}{F}$ where ΔP is the pressure gradient across the lung and represents the difference between the mean PA and the mean PV pressures and F is the flow through the lung[7]

RESULTS

Data were obtained in 20 experiments on dogs placed in the right and left lateral decubitus positions and thus the changes observed when each lung was dependent were compared with observations made when the same lung became nondependent All observations are summarized in Figure 1 and one illustrative experiment is shown in Table 1

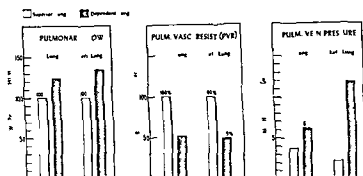


Fig 1 Graphic representation of the overall average effects of positional change on the pulmonary blood flow pulmonary vascular resistance and the pulmonary vein pressures in 20 dogs

Table 1 Data Illustrating One Experiment

PULMONARY FLOW cc /min (% of C O given)†				VASCULAR RESISTANCE mm Hg/100 cc /min (% of Sup lung given)				PULMONARY VEIN PRESSURE†† mm Hg			
RIGHT LUNG		LEFT LUNG		RIGHT LUNG		LEFT LUNG		RIGHT LUNG		LEFT LUNG	
SUPER	DEPEND	SUPER	DEPEND	SUPER	DEPEND	SUPER	DEPEND	SUPER	DEPEND	SUPER	DEPEND
1474 (46%)	2061 (68%)	991 (32%)	1744 (54%)	1.28	0 48 (37%)	1.51	0.57 (44%)	—2 0	2.5	0.5	4.5

†C O = cardiac output
††Measurements of the pulmonary vascular pressures were recorded against atmospheric pressure

Pulmonary Circulation The blood flow through each lung was calculated in each experiment and expressed as a percentage of the cardiac output Furthermore in order to compare the blood flow in the dependent versus the superior positions of each lung the flow in the dependent lung was

later expressed as a percentage of the flow in the same lung in the superior position. Thus the blood flow increased an average of 23% in the right lung (range 6 to 60%) and an average of 34% in the left lung (range 12 to 100%) when these lungs were dependent. In only 1 out of 20 cases was there a fall in the flow (2% right lung, 3% left lung). It should be noted that in all cases the increase in flow was a reflection of the increase observed in the oxygen uptake rather than any wide variation in the oxigen content of the arterial and venous bloods.

Pulmonary Vascular Resistance. It was not possible to calculate the individual pulmonary vascular resistance (PVR) in all the experiments, due to occasional difficulties in the measurements of the pulmonary vascular pressures. However, 13 calculations were possible for the right lung and 11 for the left. Expressed as a percentage of the value in the superior lungs, the average PVR fell to 51% and 49% in the right and left lungs respectively in over two-thirds or 16 of the calculations; in the remaining one-third there was a rise in the PVR in both lungs in spite of the fact that in these cases there was an increase in the blood flow.

Relation of Mean PA and Mean PV Pressures to Position Change. The mean pulmonary vein pressures (i. e., outflow pressures) in the dependent lungs were elevated in all the recordings. The average value for the right inferior pulmonary vein pressure when the right lung was in the dependent position was 6.1 mm. Hg compared with 3.7 mm. Hg when the lung was superior. Similarly, the values for the left pulmonary vein pressure were 11.9 mm. Hg (left lung dependent) compared with 2.2 mm. Hg (lung non-dependent). The overall pulmonary vein pressures were therefore elevated approximately threefold in the dependent lungs over those when the lungs were superior (9 mm. Hg versus 2.9 mm. Hg).

The pulmonary artery pressures under similar circumstances did not vary appreciably with changes in position of the lungs, the overall average in both lungs when dependent being 13.1 mm. Hg compared with 11.0 mm. Hg recorded in the superior position in the same lungs

DISCUSSION

That increase in blood flow, which at times may be considerable, takes place in a lung when it is placed in a dependent position is fairly conclusively demonstrated in these experiments. Previous authors[1, 2, 3, 4, 5] based their conclusion mainly on the fact that they observed increased oxygen consumption and only small changes in ventilation, although one[2] observed in addition a rise in the oxygen saturation of the blood in humans made to breathe nitrogen through one lung and pure oxygen through the other whenever the oxygen breathing lung was placed in a dependent (lateral decubitus) position With regard to the mechanism involved in this increase in the flow of blood, some workers[2, 3] suggested that due to gravity there was an increase in the pulmonary artery pressure in the dependent lung which accounted for the increase in flow, while others[5, 6] suggested this was due to a decrease in the vascular resistance resulting from alterations in the volume of the dependent lung Results from the present investigation are not in agreement with the above hypotheses and suggest rather that the increase in flow resulted from the fall in vascular resistance as a consequence of the marked elevation, apparently due to gravity, in the

pulmonary vein (outflow) pressures of the dependent lungs Similar observation on the inverse relationship between pulmonary vein pressure and pulmonary vascular resistance were made in 1953 by Haddy and Campbell who felt that as the pulmonary vein pressure became elevated it reduced the pressure gradient across the pulmonary vascular bed (i e, ΔP) with resultant drop in vascular resistance

The pulmonary venous bed is a nonrigid, readily distensible network with relatively little extravascular compression by the normally loose lung parenchyma Consequently, minimal elevations in the intraluminal pulmonary vein pressure will result in near maximal distension of the vascular bed Since resistance to flow varies inversely with the fourth power of the radius of a vessel it becomes readily apparent why a slight rise in pulmonary vascular pressure produces a large drop in PVR

The pulmonary artery pressures in the present experiments did not vary appreciably with changes in position and therefore are considered to play a minor role if any in the mechanism of increased flow in the dependent lung

It may be noted that application of this relationship between position and pulmonary blood flow in individual lungs is of importance clinically in determining the best positions patients are to assume in postoperative states after unilateral pulmonary resections in patients with extensive unilateral pulmonary disease, and in other situations when the healthier lung may be placed in a dependent position to best advantage Proportionately higher blood flow in the dependent lung might be expected if these experiments were conducted in humans since the transverse diameter of the chest in humans is much larger than in dogs

SUMMARY

In dogs under the conditions studied the calculated pulmonary vascular resistance in an individual lung decreased with simultaneous increase in the flow of blood whenever the lung was placed in a dependent position The mechanism for these changes is discussed and appears initially to be a rise in the pulmonary vein pressure (outflow pressure) due to gravity and subsequently a drop in the vascular resistance and therefore a resultant increase in the blood flow These steps are discussed in relation to hypotheses by other workers

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RESPIRATORY PARALYSIS FOLLOWING PULMONARY DENERVATION*

HECTOR S HOWARD AND WATTS R WEBB

In previous experiments it was noted that dogs having completely dener vated lungs, either autologous or homologous, did not resume spontaneous respiration On the other hand, dogs with only partial denervation whether with autologous or homologous lungs, appeared able to continue a normal respiratory pattern The original observations were as follows

Group 1. Perfusion Experiments. The first group had the heart and lungs totally denervated incidental to an experiment evaluating survival time of the heart deprived of circulation[1] The heart and lungs were dissected completely free from the mediastinum The inferior and superior venae cavae, aorta and trachea were isolated but were not transected After inflow and outflow occlusion was obtained, the dogs were maintained by a pump oxygenator for 90 minutes After the hearts were restored to satisfactory function with normal blood pressures and pulses, the chests were closed *The 6 dogs which survived all the other hazards of this* experiment failed to resume spontaneous respiration when taken off arti ficial respiration

Group 2. Cardiopulmonary Transplantations (*A*) A second group of 6 dogs had transplantation of the heart and both lungs from one dog to another,[2] which unquestionably produced total denervation of the lungs *The hearts functioned satisfactorily in that they maintained adequate* circulation in the recipient animal None of the dogs, however, was able to resume spontaneous respiration, even though maintained by artificial respiration as long as 22 hours

(*B*) Three other dogs had autotransplantation of their heart and both lungs After complete dissection, the major connections were severed, the heart and lungs were lifted out of the chest and then re anastomosed Though the hearts functioned well, not one of these dogs could breathe spontaneously

(*C*) In another experiment, 3 dogs had only the left lung and heart removed and transplanted to a recipient dog After discontinuance of the artificial respiration the recipient dogs which had a normally innervated right lung had a spontaneous resumption of adequate respiration

These observations are not in accord with the concept of a self sufficient medullary respiratory center with an intrinsic periodicity Further experi ments were designed to evaluate the influence of the various pulmonary neural pathways on respiration

Group 3. Pulmonary Denervations. (*A*) In 8 dogs dissections were carried out to achieve total mediastinal isolation of the heart and lungs except *that no perfusion cardiopulmonary bypass*, or transplantation was per formed This was done to rule out any deleterious medullary effects from the cardiopulmonary bypass or transplantation procedures themselves The venae cavae, aorta and pulmonary artery were freed of all surrounding

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Supported by U S Public Health Service Grant No H 2806

attachments but were not transected The trachea was freed high into the neck During the isolation of the heart and lungs all vagal and sympathetic fibers going to both structures were divided All 8 dogs died a respiratory death within minutes to a few hours after termination of the procedure Several exhibited a gasping type respiration with active expiration but none resumed spontaneous normal respiration Autopsies ruled out intrathoracic causes of death

(*B*) In 4 more dogs the chest dissection was carried out in exactly the same manner except that the dissection was not carried high on the trachea All 4 dogs resumed spontaneous respiration at the termination of the procedure This suggests innumerable interconnections between the various afferent pathways from the lung perhaps with some passing through the submucosal tissues of the trachea

Group 4 Staged Denervations (*A*) Nine dogs were operated upon as follows The chest was opened through a sternal splitting incision and the sympathetic chain was removed bilaterally from T9 or T10 to above the middle cervical ganglion The vagal trunks were then elevated and all branches to the mediastinum were divided under direct vision In some instances the phrenic nerves were stripped free of their attachments to the pericardium and pleura but in no instance were they transected or traumatized The chest was closed and then the vagus nerves were divided high in the neck At the completion of the operation all had normal blood pressures with slow bounding femoral pulses All had lid reflexes and were thought to be in light anesthesia It did not seem to matter whether pentobarbital or pentothal was used Two dogs breathed satisfactorily but 7 had immediate and progressive respiratory embarrassment Three animals made no respiratory efforts 3 others breathed at rates of 4 to 6/min for from 1 min to 2 hours One gasped 36 times per minute but died in 12 minutes If placed on artificial respiration the dogs remained in good condition during its continuation (up to 6 hours) but rapidly deteriorated when left to their own respiration function

(*B*) Six dogs were then dissected in a similar fashion except that the cervical portion of the dissection was done first As would be expected the cervical vagotomy decreased the rate and increased the volume of respiration When the stellectomy was done first the respiratory rate increased and the volume decreased much like the response obtained when a bilateral thoracic sympathectomy was performed After completion of the denervation the results were similar in that 4 of the 6 dogs died a respiratory death

Thus the order of the steps of the denervations did not seem important One of the staged denervations was done as follows A bilateral thoracocervical sympathectomy was done first and two days later a bilateral vagotomy Respiration initially slowed to 4 to 6/min but gradually increased Twenty four hours later the chest was re-opened and the vagal fibers going to both the heart and the lung were transected After this procedure the dog had a shallow gasping respiration and died a respiratory death within 2 hours In a total of 4 of the staged operations the chest was re opened for thoracic vagal stripping as the last denervation Respiratory paralysis did not occur until this had been done even though other experiments showed that a thoracic vagotomy

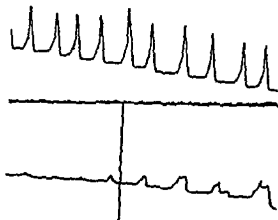


Fig 1 Total pulmonary denervation (Group 3) Control and post denervation This dog though maintained 4 hours with artificial respiration never achieved adequate spontaneous respiration Records read from right to left

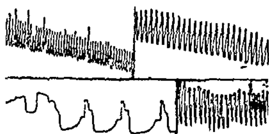


Fig 2 (Group 4) Top—right control Top—left post cervicothoracic sympathectomy Bottom—right post cervical vagotomy Bottom—left post thoracic vagal stripping Dog never returned to spontaneous respiration after these gasps though maintained for 2 hours by artificial respiration

does not materially alter the respiratory pattern produced by a cervical vagotomy This particular phase of the experiments suggests strongly that there are vagal fibers which communicate with the higher centers by a route other than those now recognized to pass through the vagal trunk in the neck

DISCUSSION

We found it extremely difficult to totally denervate the lungs and often would have to re open the chest or neck and search for missed branches Those experiments in which unequivocably the lung was totally denervated always eventuated in respiratory paralysis As the staged procedures did not produce uniform results, it seems probable that varying degrees of denervation had resulted

The Hering Breuer reflexes and other afferents from the lung have been assigned varied importance by other investigators Cromer and Ivy[3] have shown that the stellate ganglion is a sensory pathway for central reflex effects on respiration and circulation, and that the removal of the stellate ganglion sensitizes the respiratory center to vagal inhibition Chatfield and Purpura[4] report death by primary respiratory failure in 1 of 6 cervical vagotomized cats when the dorsal roots of T1, C8 or both were severed These results would likewise suggest the importance of pulmonary afferents for adequate respiration

One currently accepted theory of respiratory control is that the medullary respiratory center has an intrinsic periodicity able to maintain adequate respiration more or less independent of various afferent stimuli There is no doubt that the medullary respiratory center has the coordinating control of respiration It is responsive directly to chemical stimuli and to afferents not only from the lungs but from multiple other sources such as the cerebral cortex, innumerable proprioceptive and sensory fibers and the carotid bodies[5] All afferents have an effect on the center with resulting

modifications of respiration but it would seem that the afferents from the lung are essential for continued spontaneous respiration

SUMMARY AND CONCLUSIONS

1 Denervation of the lungs was performed in varying degrees and by many techniques Total denervation is difficult to accomplish and requires total isolation of the heart and lungs or extensive cervicothoracic sympathectomy plus cervical vagotomy and intrathoracic vagal stripping

2 Partial denervation of the lungs will alter the rate and depth of respiration but will not result in a respiratory death

3 Under the circumstances present in our various experiments complete denervation of the lungs usually results in primary respiratory death in the dog

4 Pulmonary afferent vagal fibers appear to have a central connection through pathways other than the cervical vagal trunk

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THE PREVENTION OF ALVEOLAR AIR LEAKS FOLLOWING PULMONARY RESECTION*

JAMES R CANTRELL AND GEORGE H WELCH JR

All segmental and many lobar pulmonary resections leave varying amounts of open unpleuralized surface on the remaining pulmonary tissue It is inevitable that air leaks will occur from these raw surfaces It is equally certain that the use of segmental resection will increase as pulmonary lesions are more routinely attacked in anatomical fashion and as a greater number of patients with borderline ventilatory function are operated upon It is therefore to be anticipated that the difficulties associated with postoperative air leakage will continue to pose a serious problem

Many of the postoperative complications of pulmonary resection can be directly related to this unavoidable air leakage Such leakage may result in atelectasis in the early postoperative period or in delayed or even permanent failure to achieve maximal expansion in the long term These

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are serious under any circumstances but are particularly disadvantageous in the patient who has minimal respiratory reserve, occasionally spelling the difference between survival and nonsurvival Failure of remaining lung to expand fully and thereby completely occupy the pleural space is also associated with an increased incidence of empyema The development of bronchopleural fistulae can be similarly related to such problems, this complication rarely occurring if immediate and complete expansion is obtained

The usual methods upon which we depend for obtaining early maximal expansion in the postoperative period are not ideal Reduction or elimination of air leakage would obviously be advantageous If it were possible to devise a means whereby such leaks could be prevented at the time of operation, one might expect distinct advantages to result It was the purpose of this experimental study to develop and test such a technique

METHOD

Adult mongrel dogs were used as the experimental animal Anesthesia was maintained by positive pressure endotracheal ether A pulmonary resection was designed to produce a standard large, open pulmonary surface with free alveolar air leakage The use of a formal segmental resection was discarded since it has been previously shown[1] that dogs will routinely survive this procedure whether or not special care is taken to prevent air leaks It was out intent to produce a preparation in which 100% mortality would result in untreated animals from air leakage and tension pneumothorax The procedure chosen involved resection of the entire anterolateral surface of the left diaphragmatic lobe to a depth of 2 to 3 mm, leaving a triangular raw surface with free leakage of air This defect usually measured about 5 cm on each side A typical defect with diffuse air leakage is shown in Figure 1 All vessels and recognizable bronchi were clamped and ligated so that the only air leakage was a diffuse loss from the alveoli

A control series of 16 animals was subjected to this procedure Following creation of the open surface, the chest was closed without further attention to the denuded area Closure was performed in routine fashion, positive pressure being discontinued as soon as air tight closure was effected In a second series of 22 animals in which a similar resection was employed the raw surface was immediately covered with powdered gelatin sponge



Fig 1 The denuded open pulmonary surface showing free air leaks



Fig. 2. The same surface after being covered with a thin layer of powdered gelatin sponge. All air-leakage has ceased.

(Gelfoam†). In 10 animals of this group, the gelatin sponge was applied as a dry powder which adhered to the serum and blood on the raw surface, forming a thin eschar (Figure 2). In 12 animals of the group, the Gelfoam was first mixed with autogenous blood to form a paste, a thin film of which was spread on the open surface. The results in these subgroups were identical so that they will be considered as a single category. Neither chest drainage nor suction was utilized in the postoperative care of either the control or the experimental group. Thoracenteses were not employed and no antibiotics were given to either group.

RESULTS

It has been observed that in the dog, whose mediastinum is extremely mobile, a minute but persistent air-leak will result in severe tension pneumothorax and death. Evaluation of the effectiveness of the technique was based upon the ability of the technique to prevent death from tension pneumothorax.

In the control series, 13 of 16 animals (81%) died, death invariably resulting from tension pneumothorax and usually occurring within 30 minutes of chest closure. In the group in which powdered gelatin sponge was applied, only 3 of 22 animals (13.6%) died of tension pneumothorax. The difference in the mortality in these groups is statistically significant. Expansion of the remaining lung was usually complete by the second or third week despite the fact that pleural aspiration was not utilized in order to assure each expansion.

One animal of the treated group died of distemper 8 days following operation and at autopsy was found to have an empyema on the operated side. No other animals developed thoracic infections of any kind.

Pathology. The treated animals were sacrificed at intervals ranging from 3 days to 7 months. Specimens were examined grossly and microscopically.

Gross Observations: During the first week, the lung surface appeared hemorrhagic, dull and opaque. The lung did not fully expand and patchy areas of atelectasis were noted. During the next two weeks, the lung reached full expansion and the eschar gradually disappeared, leaving the operated lung surface covered by a thin layer of opaque tissue which had the gross appearance of thickened pleura. After a period of months, this

†Upjohn Company, Kalamazoo, Mich

layer became quite thin and only slightly more opaque than normal pleura. There was no significant contracture of the pulmonary tissue. A minimal number of adhesions were noted between the parietal pleura and all lobes. These were neither dense nor extensive.

Microscopic Observations: at 72 hours, marked capillary dilatation was noted with exudation of fluid into tissue spaces and alveoli. Some hemorrhage into the alveoli was observed but this was not extensive. The wound surface itself was coated by the gelatin sponge permeated with fibrin with surprisingly few recognizable blood elements. Over the course of the next 10 to 12 days, the parynchemal reaction resolved so that by the end of two weeks, the underlying pulmonary tissue appeared essentially normal. The eschar of fibrin and gelfoam either separated or was absorbed so that at two weeks the surface was covered by a relatively thin layer of fibrous tissue overlying an abundant vascular network. It would appear that the healing process takes place beneath, rather than by organization of, the eschar so that no trace of the gelatin sponge can be found in those animals examined after the healing process is completed.

It is interesting to note that the superficial connective tissue layer is not greatly thicker than the normal pleura and that it appears to be covered by a layer of mesothelial cells similar to the normal serosal covering. The cells are slightly larger and are much closer together than the serosal cells covering normal lung.

DISCUSSION

The place of segmental pulmonary resection in the techniques of thoracic surgery is accepted. The problems associated with residual open pulmonary surfaces must also be accepted. The standard method of dealing with air leaks from such surfaces consists of the use of water sealed drainage or suction, drawing off the air which accumulates in the pleura but making no attempt to stop or prevent the leaks themselves. Air leakage is accepted as a necessary and unavoidable evil until such time as the normal healing process obliterates the small alveolar openings, at which time the lung will expand maximally. This interval may be uncomfortably long and may even result in permanent failure of the lung to expand optimally.

It appears[1, 2] that the closure of these leaks is normally accomplished by diffuse exudation of fibrin from the wounded surface. This fibrin forms an eschar over the injured area, filling the open alveoli and obliterating the small alveolar pleural fistulae. The present technique was designed to mimic this process as nearly as possible but in such a fashion that the end result, i. e., closure of these fistulae, could be accomplished at the time of operation rather than 48 or more hours later.

Various methods have been advocated for the control of alveolar air-leakage from open lung surfaces. Valid objections may be raised to many of these, especially those which advocate formal closure of the defect, thereby producing distortion of the bronchovascular structures with consequent ventilatory dysfunction. The present method does not suffer from this difficulty and does not appear to result in late distortion as the result of scar contracture. This finding is in disagreement with the conclusions of Kausel and Lindskog[1] based on limited experiments with Gelfoam utilizing a different technique. Neither does the method suffer from the

difficulties which might be encountered in those techniques which attempt to seal leaks by suture of adjacent pulmonary tissue or a free flap of pleura over the open surface The possibility of loculation of air beneath such a closure is apparent

Gelatin sponge was utilized because of its proven innocuous characteristics in the human and its tendency to more or less complete absorption The powdered form was utilized because it allowed the application of a very thin yet effective layer of material and because of its ability to conform to an irregular surface No complications were noted which could be attributed to the use of this material

The method would appear to be highly efficacious in preventing alveolar air leaks It does not appear to predispose to infection or to interfere grossly with ventilatory function Healing of the open lung surface appears to progress essentially as in the untreated open pulmonary wound

The method is currently being tested in the human

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THE REVERSIBILITY OF CHRONIC ATELECTASIS*

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EDWIN T LONG, GERALD P HERMAN, AND WILLIAM E ADAMS

Chronic atelectasis may be secondary to bronchostenosis of traumatic, congenital or tuberculous origin Reports of resection of the stenotic portion of the bronchus with reinflation of the lung are not infrequent in the recent literature, although there has been strikingly little evidence that chronic atelectasis is a reversible process If it can be shown to be reversible, bronchoplasty and reinflation is the treatment of choice If not reversible then reinflation of the atelectatic lung may actually be harmful to the patient for it has been shown that an expanded nonfunctioning lung is a greater physiological deficit than a collapsed lung [1] Accordingly, the following series of experiments was undertaken to gain additional information as to whether the atelectatic lung should be removed or reinflated

*From the Department of Surgery and Physiology of the University of Chicago Supported in part by United States Public Health Grant No H 1894 (C7) and in part by the E F Andrews Fund for Thoracic Surgery

With the technical assistance of Mr Hilbert Dotson

METHOD

Atelectasis of the entire left lung was produced in 15 mongrel dogs by the transbronchoscopic application of 35% silver nitrate solution circumferentially to the mucosa of the left mainstem bronchus.[2] One or two applications usually sufficed to produce the desired complete stenosis which occurred 10 to 14 days after cautery.

The diagnosis of atelectasis was made by physical examination, bronchoscopy, roentgenograms and bronchograms. A typical roentgenogram showed shift of the mediastinum to the atelectatic side. The diagnosis was later confirmed at thoracotomy.

The physiological deficit produced by the atelectasis was evaluated by means of graphs relating arterial oxygen saturation to alveolar oxygen tension as described by Perkins, *et al.*[3] Arterial oxygen saturations were determined by means of a modified Wood continuous recording cuvette oximeter which had been calibrated with Van Slyke-Neill determinations Blood from the femoral or carotid artery was circulated through the cuvette and returned to a convenient vein, clotting being prevented by the administration of heparin. End expiratory samples approximating the composition of alveolar air were obtained with a Rahn valve, and analyzed by Pauling-Beckman model C oxygen analyzers. Mixed venous blood was obtained from a cardiac catheter fluoroscopically positioned in the pulmonary artery or right ventricle.

The vessels used for the cuvette, and the jugular vein used in introducing the cardiac catheter were exposed using procaine infiltration anesthesia. In this way it was possible to determine the peripheral arterial saturation and mixed venous saturation with the animal awake and unsedated, breathing room air.

Anesthesia was then induced using 12 to 17 mg./kg. of intravenous pentothal. While administering this it was possible to monitor the saturation with the oximeter, thereby making certain that it was maintained at the pre-anesthetic levels. A saturation-tension curve was then determined by allowing the animal to breathe varying mixtures of nitrogen, air and oxygen prepared in a Tissot spirometer. In this manner the alveolar oxygen tension was varied from 50 to 670 mm. Hg.

The fraction of right to left shunting; *i.e.*, the percentage of total cardiac output passing through the atelectatic lung, was calculated by the standard mixing equation expressed in terms of percent saturation, neglecting small amounts of dissolved oxygen. Expressed in standard terminology, the equation is: $\dot{Q}va/\dot{Q}t = (S'c_{O_2} - Sa_{O_2}) / (S'c_{O_2} - S\bar{v}_{O_2})$, where $\dot{Q}va/\dot{Q}t$ = fraction of cardiac output shunted, (X 100 = percentage of shunting), $S'c_{O_2}$, Sa_{O_2}, $S\bar{v}_{O_2}$ represent oxygen saturation of lung capillary blood, arterial blood, and mixed venous blood respectively. $S'c_{O_2}$ may be taken as 97% when the animal is breathing room air.

Within one to two weeks following the above described studies, thoracotomy was performed and the left lung was found shrunken to less than 1/4 normal size with the color and consistency of normal liver. The stenotic portion of the left mainstem bronchus was resected and anastomosis was performed with 5-0 silk utilizing a continuous everting mattress technique interrupted 4 times about the circumference of the bronchus. A

series of experiments in this laboratory, as well as studies by Jackson *et al*,[4] showed the everting method of suturing to be one of the most satisfactory for bronchial anastomosis Upon opening the bronchus of the atelectatic lung a pale viscoid mucus was constantly encountered which was aspirated as completely as possible The lung expanded easily in all cases where satisfactory anastomosis was possible

Ten days to 6 weeks after bronchoplasty peripheral arterial saturation mixed venous saturation and a saturation tension curve were again determined by the methods described previously

RESULTS

Control studies were done on 7 normal animals before producing atelectasis and normal curves lying close to the *in vitro* dissociation curve were obtained as described previously[3] Hence control studies were not carried out in all animals

Fifteen dogs with atelectasis of the entire left lung of 2½ to 32 weeks duration were studied and the physiological deficit expressed in terms of percentage of cardiac output passing through the atelectatic lung These results are summarized in Table 1

Table 1 Chronic Atelectasis Entire Left Lung (Animal Awake)

DOG	DURATION IN WEEKS	PERIPHERAL ARTERIAL SAT	MIXED VENOUS SATURATION	PERCENT SHUNT
296	2½	90	49	12
998	3½	87	59	26
271	5	86	58	28
34†	7	82	54	33
589	7	95	67	7
961	7	95	58	5
297	11	86	47	22
309	15	93	54	9
113	15	95	57	5
939	19	94	75	14
189	19	91	58	15
333	20	99	54	0
278	26	95	54	5
822	27	88	57	22
124	32	87	47	20

†Atelectasis plus left pneumothorax

Shunting in the 14 dogs who had uncomplicated atelectasis of the entire left lung varied from 0 to 28% with an average value of 14% In two of the animals Dogs 998 and 822 the determinations were repeated on different days and the original values were confirmed within the limits of accuracy of the method

Successful bronchoplasty i e complete reinflation of the lung with permanent establishment of an adequate bronchial lumen as judged by bronchoscopy roentgenograms and bronchograms was accomplished in 9 of the 15 animals The most common reason for failure being a stenotic segment too long to allow ideal anastomosis The chronically atelectatic lungs reinflated to only approximately ⅔ normal volume except when unusually high positive pressure was applied Repeat studies after reinflation of the atelectatic lungs demonstrated marked physiological improvements as summarized in Table 2 Saturation tension curves of one animal are presented in Figure 1

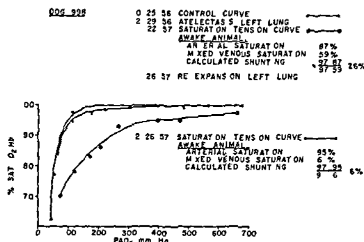


Fig 1 The Control saturation tension curve obtained on the animal prior to production of atelectasis closely approximates the *in vitro* oxygen dissociation curve for the blood of dogs The lower most curve was determined 3½ weeks after the development of chronic atelectasis *showing the depression and* linearly rising slope characteristic of anatomical right to left shunting The intermediate curve obtained one *month after reinflation of the* atelectatic lung approaches the Control curve—signifying a marked reduction of right to left shunting

Table 2 Chronic Atelectasis Entire Left Lung (Results of Bronchoplasty)

DOGS	DURATION IN WEEKS		PERCENT SHUNT	
	ATELECTASIS	REINFLATION	ATELECTASIS	REINFLATION
296	2½	3½	12	0
998	3½	4	26	6
31	7	9	33	0
961	7	8	5	0
309	15	19	9	0
939	19	22	14	4
189	19	20	15	0
822	27	29	22	0
124	32	32	20	2

†Atelectasis plus left pneumothorax

DISCUSSION

A number of previous investigators have demonstrated that blood flow through the atelectatic lung is markedly reduced[5 6 7 8] Moore[6] showed that the ratio of the blood flow through the right lung to that in the left in the normal dog is 3 2, and in studies on acute atelectasis with the chest closed in anesthetized animals found an average of 17 5% of the cardiac output passing through the atelectatic left lung Keeley[7] studying acutely and chronically atelectatic lung in dogs with the chest open found an average of 15 6% shunt The present study revealed a blood flow ranging from 0 to 28%, and averaging 14% of the cardiac output through atelectatic lung of 2½ to 32 weeks duration The degree of shunting did not appear to be appreciably different between our "long term' and 'short term' animals Therefore the results of our study compare closely with those of previous workers in spite of the elimination of the variables of anesthesia and open chest preparations

Previous studies from this laboratory,[9 10] as well as those of Webb and Burford,[11] have failed to delineate any changes in the microscopic morphology of the chronically atelectatic lung or such a lung reinflated Grossly no difference was noted in the ease with which the 'long term" and "short term' atelectatic lungs reinflated at surgery Following successful bronchoplasty the average 'shunting' was reduced to approximately 1%, signifying a marked improvement in function as compared to the studies prior to reinflation

The question as to whether the function of these reinflated lungs is adequate to solely support an animal s life on a long term basis is under investigation at present Preliminary evidence would indicate that this function does not immediately return fully and that much of the abnormality of the reinflated chronically atelectatic lung is due to increased pulmonary vascular resistance with resulting pulmonary hypertension

SUMMARY

Atelectasis of the entire left lung was produced in 15 dogs by chemical cautery of the left mainstem bronchus After 2½ to 32 weeks of atelectasis, physiological deficit was measured in the awake unsedated animal with an intact chest with the aid of a continuous recording oximeter An average of 14% of the cardiac output was found to be flowing through the atelectatic lung The stenotic segment of bronchus was later resected, the lung reinflated and the animal was then again evaluated physiologically Postoperatively an average of 1% shunting was found, representing significant improvement in pulmonary function with reinflation of the atelectatic lung

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ANESTHETIC CONVULSIONS THE ROLE OF ETHER AND HYPERTHERMIA IN THEIR PRODUCTION*

GUY OWENS, ROYCE E DAWSON, AND H WILLIAM SCOTT, JR

Convulsions which occur during surgical anesthesia are rare This is tunate because permanent neurologic defects and even death may foll Recent personal experience with these anesthetic complications plus in mation gleaned from the literature suggested that a combination of hy thermia and ether anesthesia were major factors in the development these phenomena [1 2 3 4] Since this hypothesis needed experimental eva tion and because information concerning the prevention of anesth convulsions was inadequate, this study was undertaken

METHOD

Sixty five adult mongrel dogs were used in this study Using continu electroencephalographic and electrocardiographic monitoring, the eff of artificially elevated body temperature were assessed prior to or dur exposure to a variety of anesthetic agents These included diethyl et divinyl ether pentothal, pentothal plus ether, chloroform, cyclopropa ethylene and nitrous oxide Hyperthermia was induced by the use o heated rubber blanket and levels of body temperature ranged from 1 to 108°F In those animals made hyperthermic prior to anesthetizat and in the group which served as hyperthermic controls succinylchol was administered intravenously in a saline drip to produce immobil The various inhalation anesthetic agents were given via a closed syst in which a soda lime CO_2 absorber was incorporated All animals w intubated and breathed, or were manually assisted in breathing, 10

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oxygen Blood ether levels as well as random samples for pH CO_2 and glucose determinations were obtained Microscopic studies were made of sections of brains removed from animals which developed neurologic deficits convulsions or died as a result of the experiment

RESULTS

Diethyl ether was administered to 10 animals 5 of which were made hyperthermic during and 5 prior to anesthetization with this agent In each instance severe EEG abnormalities were produced Three of 5 animals in the first group demonstrated EEG recorded seizures at temperatures ranging from 105 to 107°F Initial abnormalities were observed at 102 to 103°F and consisted of bursts of 2 to 3/sec waves ranging in amplitude from 150 to 200 microvolts As the temperature increased these bursts became more frequent until the record became filled with abnormal wave forms or until a seizure response was noted (Fig 1) The five animals made hyperthermic (105°F) prior to anesthetization developed EEG recorded seizures in each instance 1 to 3 minutes following ether inhalation Blood ether levels at the time of the seizures in these latter animals ranged from 35 to 45 mg% This is well below the ether level of 100 mg% necessary for muscle relaxation The seizures could not be abolished by increasing the depth of anesthesia even to the point of severe cardiac irregularities Random sampling of blood pH CO_2 and glucose levels indicated that the deviations from the normal were similar to the changes expected in dogs with ether anesthesia alone

The five animals from the first group all survived the experimental procedure but 4 developed varying degrees of neurologic deficits including gross tremor of all extremities and head loss of equilibrium and a defect in coordination Some correlation existed between the severity of the EEG change and the degree of neurologic involvement Three of the animals most severely afflicted were sacrificed at 4 6 and 14 days Grossly the brains were normal A generalized mild neuronal involvement was seen with changes at the fourth and sixth day consisting of clumping of the Nissl substance and pyknosis In the animal sacrificed at 2 weeks almost no Purkinje cells remained in the cerebellar cortex There appeared to be a minimal reduction of neurons in this animal in the basal ganglia as well as cerebral cortex No phagocytosis was identified The myelinated

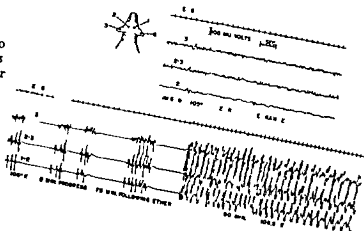


Fig 1 Electroencephalographic tracing of seizure discharges in dog during ether (diethyl) anesthesia and associated hyperthermia

structures and long fiber tracts of the various brains examined appeared uninvolved

Four of the 5 animals made hyperthermic prior to ether inhalation died during the experiment The fifth animal died 8 days later from unknown causes Examinations grossly and microscopically of the brains from these animals were within the limits of normal

Five animals used as hyperthermic controls (106 to 108°F) while immobilized by succinylcholine did not develop abnormal electroencephalographic wave forms or seizure activity during periods of 1 to 2 hours These animals recovered without neurologic disturbances

In 10 dogs which were anesthetized with divinyl ether after and during induced hyperthermia seizure activity was recorded by the electroencephalograph in each instance In 3 of these animals seizure patterns appeared prior to temperature elevation and persisted for the duration of the hyperthermic period In the 7 other dogs in this group the abnormalities were evident at temperatures of 105 to 108°F In those animals made hyperthermic prior to inhalation of divinyl ether the seizure record appeared within 1 to 3 minutes after introduction of the drug No evident neurologic damage was produced in these 10 animals and microscopic examination of their brains at various time intervals (1 to 6 weeks) after anesthesia disclosed no detectable pathologic alterations

Table 1 summarizes the experience with the other anesthetic agents which were studied in animals with induced hyperthermia (105 to 108°F) No gross or recorded seizures occurred in any animal anesthetized with pentothal, chloroform, cyclopropane, ethylene or nitrous oxide and no evidence of damage to the central nervous system was found It is note

Table 1 CNS Responses in Dogs to Hyperthermia and Various Anesthetic Agents

AGENT	NUMBER OF DOGS	EEG ABNORMALITIES SEIZURES	EEG ABNORMALITIES OTHERS	CNS DEFICITS	DEATHS	MICROSCOPIC BRAIN ABNORMALITIES
Ether (diethyl)	10	8	2	4	5	Present
Divinyl Ether	10	10	0	0	0	Absent
Pentothal	5	0	0	0	0	-
Pentothal + Ether	5	0	0	0	0	-
Chloroform	10	0	1	0	0	Absent
Cyclopropane	10	0	0	0	0	
Ethylene	5	0	0	0	0	-
N_2O	5	0	0	0	0	
Succinylcholine Immobilization (control)	5	0	0	0	0	-

worthy that in 5 animals anesthetized with pentothal and rendered hyper thermic the addition of diethyl ether for periods of 30 to 60 minutes failed to produce the changes previously observed with ether and high temperature alone An abnormal EEG tracing without seizures occurred in one of the 10 animals which received chloroform No neurologic changes were pro duced however and microscopic examination of the brain of this dog was negative

DISCUSSION

In attempting to define the parameters of the problem of anesthetic convulsions the information obtained in this study tends to support the specific accuracy of the commonly used term ether convulsions Under the circumstances of these experiments ether compounds in association with hyperthermia have consistently produced electroencephalographic sei zures but have demonstrated variations in toxicity to the central nervous system Only diethyl ether produced both seizures and permanent neuro logic damage with identifiable brain lesions Divinyl ether was capable of producing electroencephalographic seizures but these were not followed by clinical gross or microscopic evidence of central nervous system damage

The phenomena produced experimentally in dogs by a combination of diethyl ether and hyperthermia appear to be identical with those observed in patients with ether convulsions The protection offered by pentothal to hyperthermic animals anesthetized by that agent when ether is sub sequently added for periods up to one hour provides experimental support for the use of barbiturates in the control of ether convulsions clinically Prevention of the convulsions however is the important goal since they reflect cellular damage in the brain Electroencephalographic warning is possible as shown by these experiments and with increasing use of this instrument in clinical anesthesia prophylactic efforts should be more successful It is obvious that ether compounds should be avoided as anes thetic agents in patients with high temperatures The other agents included in this study (with the exception of chloroform in an isolated instance) were tolerated without any evidence of abnormal developments

The specific changes which take place in the biochemical processes of the central nervous system have not been identified The complexity of the problem at the biochemical level is recognized but by pursuing it some light may be shed on the specific role of ether as an anesthetic agent

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VENOUS PRESSURE AND CARDIAC EFFICIENCY DURING ANESTHESIA*

RICHARD C. MCPHERSON, ERIC OGDEN, JOHN R. JONES, AND JAY JACOBY

Engorgement of superficial veins develops in many patients following induction of anesthesia. This fact is utilized when it is necessary to place large needles in veins prior to major surgery. Reports of venous pressure changes during anesthesia have been conflicting, and have not adequately explained the phenomenon.[1, 2, 3, 4, 5, 6]

Some Studies of Circulation in Man. Adult patients who had no abnormalities of the cardiovascular system were selected. The usual technique of balanced anesthesia was followed. Sedation was accomplished with meperidine and scopolamine. Anesthesia was begun by the administration of pentothal, 50 mg. every 15 seconds until light surgical anesthesia was reached. Six to 15 mg. of d-tubocurare were administered in divided doses and inhalation anesthesia was maintained using oxygen with cyclopropane, nitrous oxide or ether. Endotracheal tubes were not used nor was artificial respiration. These patients breathed spontaneously, with no obstruction and no straining.

Observations were made on 27 patients following the sedation, 5 to 10 minutes after induction when the patient was in light surgical anesthesia, and at the conclusion of the operation while the patient was still fully anesthetized. The observations included peripheral venous pressure, ballistocardiogram, blood pressure, heart rate, and oscillometry. The work of the heart, the stroke volume and cardiac output were calculated.[7]

Findings. During the period of anesthesia, 92% of patients had increases in venous pressure ranging from 15 to 195 mm. of water. The mean venous pressure was 95 and it rose to 136 mm. of water. In only two cases was there a slight fall. In one third of the patients the venous pressure gradually returned toward normal, but in only one case was the previous level reached.

There were no significant changes in cardiac output, stroke volume, blood pressure, heart rate, work of the heart, or oscillometry. These findings are summarized in the following table.

Table 1. Average Values for Circulatory Changes During Anesthesia

	VENOUS PRESSURE MM. H₂O	CARDIAC OUTPUT ML./MIN.	STROKE WORK GM. M./BEAT	MEAN ARTERIAL PRESSURE MM. Hg	HEART RATE BEATS/MIN.
Before Anesthesia	95	3085	47.8	96.6	80
Early in Anesthesia	136	2910	45.9	93.3	88
Late in Anesthesia	140	3200	48.9	101.5	90

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Animal Studies In searching for a cause for elevated venous pressure changes in the heart and its work capacity must be considered A drug is said to have an inotropic effect if it changes the efficiency of the heart A positive inotrope causes greater efficiency and a negative inotrope causes a reduction of the efficiency of cardiac contraction Pentothal cyclopropane and ether used individually have been shown to have negative inotropic effects while nitrous oxide without hypoxia has no inotropic effect [5 8 9 10 11 12 13 14]

Since present day anesthesiology is characterized by the frequent use of combinations of anesthetic agents the effects of such combinations were studied in nine dog heart lung preparations

The technique was that described by Patterson [15] with constant temperature and constant input load Left ventricular output was measured directly and the rate of work of the heart was calculated by the Evans formula [16]

Unpremedicated dogs were anesthetized with cyclopropane and oxygen to set up the heart lung preparation An infusion of glucose and insulin was delivered into the artificial circulation as recommended by Bayliss [17] After the artificial circulation was begun 100% oxygen was given in each experiment during the control periods The anesthetic gases were then administered by deep positive pressure artificial ventilation using a large reservoir in order to assure rapid equilibration between the gas and the blood The gases were used in doses corresponding to those administered to humans Pentothal was administered in 40 mg amounts either just before or just after equilibration with the gas The effects on the rate of cardiac work could then be compared for cyclopropane alone pentothal alone nitrous oxide alone cyclopropane in addition to pentothal and nitrous oxide in addition to pentothal

Some experiments were done at high and some at low work loads to be comparable with clinical situations and to insure that a trifling effect would not be unduly magnified in the overloaded heart

Findings The administration of cyclopropane in the low work range produced minimum negative inotropic effects indicated by a slight rise of the venous pressure In the high work range the effect was greater Similar results followed the administration of pentothal alone

Nitrous oxide either alone or in addition to pentothal had no inotropic effect

The combination of pentothal and cyclopropane produced a clearly exaggerated negative inotropic effect approximately three times as great as either one alone When the work of the heart was at a high level a single dose of pentothal following cyclopropane anesthesia was sufficient to produce a noticeable negative inotropic effect When the work of the heart was at a low level pentothal occasionally had to be repeated once or twice in order to produce a measurable effect

DISCUSSION

The preponderance of evidence in the literature indicates that the venous pressure is elevated during anesthesia Any explanation of the observed rise in the venous pressure must take into account such factors as pre

medication, room temperature, rate of intravenous infusion, and technical difficulties with the anesthesia In this study all of these factors were kept constant, and no patient was included in whom anesthetic difficulty was encountered

An accumulation of carbon dioxide in the blood, and changes in the intrathoracic pressure may influence the venous pressure [6, 18] In this study light anesthesia, a clear airway, and spontaneous breathing were utilized in order to determine what happens in the usual anesthetized patient, without complications and without interference by the anesthesiologist

Among the possible causes for elevated venous pressure are increased external pressure on the vessel, contraction of the muscular walls of the vessel, an increased amount of blood within the vessel, or a combination of these

Because these patients had muscular relaxation achieved by both anesthesia and curare, increased external pressure on the veins seems unlikely Because the veins were visibly enlarged, constriction may be ruled out An increased amount of blood within the vessels, therefore, seems to be the most logical explanation for the elevated venous pressure

Since the total blood volume does not increase during anesthesia, the increase in venous pressure and visible engorgement of the veins indicates that there is a shift of blood from some other part of the body [19] Such a relationship between the splanchnic area and the extremities has been demonstrated [20]

In accordance with Starling's law of the heart, increased venous pressure indicates that there is an increased load of work on the heart, or that the heart is becoming inadequate, or both In our patients the work load was not increased, nor was the cardiac output significantly changed If the heart were not affected by the anesthetic, an increase in cardiac output would be expected when the venous pressure became elevated Since this increase in cardiac output did not occur, the anesthetic agents must have acted as negative inotropes for the human heart *This corresponds to what has been shown for the dog heart*

CONCLUSIONS

1 For the isolated dog heart nitrous oxide and oxygen have no inotropic effect Cyclopropane alone has a weak negative inotropic effect *Pentothal alone has a moderate negative inotropic effect*

2 The combination of cyclopropane and pentothal exaggerates the negative inotropic effect

3 Interference with the efficiency of the heart by anesthetic agents becomes greater as the work load approaches the limit of cardiac capacity

4 A rise in venous pressure occurs in the majority of patients following *induction of anesthesia This is accompanied* by visible engorgement of the vessels, but is not accompanied by any consistent change in cardiac output, arterial blood pressure or cardiac work

5 The rise in peripheral venous pressure appears to be due to the negative inotropic effects of the anesthetic agents and to a redistribution of the blood, a greater proportion being in the extremities

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FUNCTION OF THE REGENERATING THYROID GLAND*

FUN LIN FONG NORMAN KALANT HARRY C BALLON, AND MARTIN M HOFFMAN

Following subtotal thyroidectomy the thyroid remnant undergoes hyper plasia and an increase in functional activity [1,2] The purpose of the present work was to study the sequence of such changes and to determine some of the adjustments to the alterations in thyroid function

METHOD

Male rats weighing 200 to 250 gm were subjected to partial thyroid ablation The size of the remnant calculated as the difference between the weights of the excised contralateral lobe and the excised portion of the ipsilateral lobe was approximately 20% of the initial weight of the gland At intervals of 5 to 75 days after surgery measurements were made in different groups of animals of the radioiodine uptake, oxygen consumption [3]

*From the Departments of Experimental Surgery and Investigative Medicine McGill University and the Departments of Surgery and Medicine Jewish General Hospital Montreal Aided by a grant from the Cancer Research Society Montreal.

and serum protein bound iodine (PBI), and the results compared with those obtained in intact animals The weight of the remnant and its *histologic characteristics were also determined at these times In addition* the effect of 1 triiodothyronine on the iodine uptake by the remnant was studied This material was given intraperitoneally in a dose of 2 5 ug/100 gm metabolic mass (body weight), daily for 5 days prior to administration of the test dose of I^{131} The effect of such treatment on the uptake by *intact rats* was measured for comparison

Animals were maintained in a room at 78°F, 40% relative humidity and constant lighting from 7 A M to 7 P M They received Purina Fox Chow and tap water *ad lib* except for the 5 days prior to administration of tracer doses of I^{131}, when they received a diet containing 140 ug of iodine/kg and distilled water Thyroidal radioactivity was measured in the excised gland in a well counter 8 to 12 hours after an intraperitoneal injection of 15 uc of I^{131}

A group of patients who had undergone partial thyroidectomy 1½ to 10 years previously were also studied All were euthyroid at the time of examination as shown by clinical state and serum cholesterol and PBI concentrations, none had had medication with iodide or thyroid extracts They were given a tracer dose of 10 uc of I^{131} and the uptake measured at 24 hours Triiodothyronine was then given in a daily dose of 50 ug for 5 days and I^{131} uptake measured on the sixth day A week later they were given intramuscular injections of thyrotrophin 5 USP units on 2 consecutive days following which the 24 hour uptake was again determined

RESULTS

Animal Studies The thyroid remnants had increased detectably in size

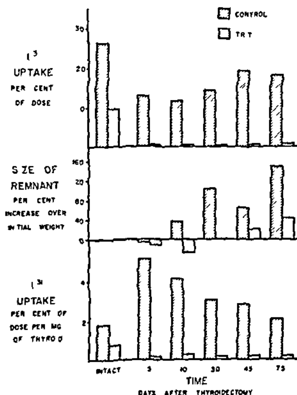


Fig 1 Changes in I^{131} uptake and weight of remnant following partial thyroidectomy and the influence of triiodothyronine

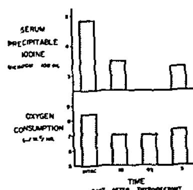


Fig 2 Changes in serum protein bound iodine and in oxygen consumption after partial thyroidectomy

by the tenth postoperative day, and continued to enlarge thereafter By the 75th day they had increased by approximately 150% Marked hyperplasia with papillary formation and absence of colloid were the characteristics of the remnants 5 and 10 days post-operatively Subsequently, there was a gradual decrease in hyperplasia with fewer papillary projections, a decrease in cell height and increasing colloid storage

The total I^{131} uptake fell by approximately 50% five days after surgery It then slowly increased, but had returned only to 72% of normal by the 75th postoperative day The uptake per unit weight of thyroid tissue, however, was increased three fold soon after operation, then declined steadily to a normal value

The serum PBI concentration dropped by approximately 30%, and the oxygen consumption rate fell by 18% following surgery and had returned only partly to normal levels after 75 days

In intact animals administration of triiodothyronine resulted in a 65% suppression of thyroidal I^{131} uptake Following partial thyroidectomy, suppression induced by the same dose was virtually complete The thyroid remnants of treated animals weighed much less than those of untreated rats and had the histologic features of a normal resting gland, with low follicular epithelium and abundant colloid

Human Studies The results obtained in 21 subjects are shown in Table 1

Table 1 Effect of Triiodothyronine and Thyrotrophin on Thyroid Function 1½ to 10 Years Following Partial Thyroidectomy

REASON FOR THYROIDECTOMY	TRIIODOTHYRONINE			THYROTROPHIN		
	NO	SUPPRESSED†	NOT SUPPRESSED	NO	STIMULATED††	NOT STIMULATED
Nontoxic Nodular Goitre	14	11	3	12	8	4
Hyperthyroidism	7	5	2	5	0	5†††

†Criteria of suppression—uptake less than 20% or reduced to 60% or less of initial value if this was low[4]

††Criteria of stimulation—uptake increased by 10% of dose or 100% of initial value if this was low

†††Four out of five had uptake diminished by 25 55% of initial value

DISCUSSION

Regeneration of the thyroid was manifested in the present experiment by a progressive increase in weight of the remnant and histologically by

epithelial hyperplasia of the follicles Coincidental with this there was a marked increase in functional activity reflected in the high uptake of radioiodine/mg of tissue This however, was apparently insufficient to meet body requirements since there was a fall in serum PBI and in the rate of oxygen consumption

As hyperplasia decreased with time and colloid formed an increasing proportion of the gland mass, accumulation of I^{131} per unit mass fell The total I^{131} uptake and the oxygen consumption rate returned toward normal while the PBI remained low The low uptake and serum PBI values are compatible with a euthyroid state if compensated by increased turnover rates of thyroidal and serum hormonal iodine[5] However, the depression of oxygen consumption indicates that even if present such compensation was inadequate in the present experiment

The dependence of regeneration on pituitary thyrotrophic hormone is seen in the effects of administration of triiodothyronine, this caused inhibition of thyrotrophic secretion and resulted secondarily in a decrease in thyroid hyperplasia and iodine uptake

Werner[4] has shown that the remnant following thyroidectomy for Grave s disease may not be inhibited by the administration of triiodothyronine This suggests that the remnant may be partly autonomous and not wholly dependent on thyrotrophin In the present small series failure of inhibition was as prevalent in the patients who had had nontoxic goiter as in those who had had thyrotoxicosis of 5 patients who failed to respond 4 had initial uptakes of less than 15% On the other hand thyrotrophin increased the uptake in 2/3 of the nontoxic group, but in the toxic group it failed to increase, and actually diminished the uptake The explanation for the latter phenomenon is not clear

SUMMARY

Following subtotal thyroidectomy in rats the thyroid remnant underwent hyperplasia and an increase in function which was insufficient to maintain a normal concentration of serum PBI or a normal oxygen consumption rate With time the hyperplasia subsided but parameters of thyroid function remained at levels somewhat below normal Regeneration and function were dependent on pituitary thyrotrophin The I^{131} uptake by thyroid remnants in human subjects was suppressed by triiodothyronine in most instances thyrotrophin increased it in most of those who had not had hyperthyroidism but diminished it in those who had

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17 21 HYDROXYCORTICOSTEROIDS IN LABOR AND DELIVERY*

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Previous studies on 17 21 hydroxycorticosteroids in obstetrical patients have shown that the blood levels of these substances rise in the antepartal period and return to normal after delivery High levels have also been demonstrated during labor by Gemzell[1,2,3] and Bayliss[4] Prior to initiation of this study, previously reported observations have usually been on the basis of one determination during labor The object of the present investigation was to determine the plasma levels of 17 21 hydroxycorticosteroids at specific times during and after the physiological stress of labor and delivery

METHOD

Fifteen normal women were selected at random from those attending the antepartal clinic at the University Hospital Their average age was 23 5 years There were 3 primigravidae and 12 multigravidae The average gravidity was 3 3 The antepartal course of all the women had been normal up to the time of their selection for the study and continued so in all cases except for one patient who went into labor prematurely at the 36th week of pregnancy Labor began spontaneously in all instances and there were no complications during labor and delivery The average duration of the first stage was 8 5 hours and of the second stage 35 minutes Minimal analgesia and anesthesia were necessary Seven patients received only trilene, self administered, during contractions Five patients received, in addition, 50 mg of meperidine Two patients received 150 and 200 mg of meperidine respectively in 50 mg doses given 3 to 4 hours apart as well as trilene Spontaneous vaginal delivery occurred in all these 14 patients, episiotomy being performed in 5 under local anesthesia with 1% procaine One primigravida received 125 mg of meperidine in divided doses and was delivered by low forceps under saddle block spinal (pontocaine) with an episiotomy The infants were normal in all instances, weighing from 4 pounds, 9½ ounces to 8 pounds, 1 ounce and none required resuscitation The immediate postpartum course of all the mothers was uncomplicated

Initial blood samples were drawn from an antecubital vein at about the 36th week of pregnancy Further samples were taken in a similar manner during active labor (6 to 7 cm cervical dilatation), at the time of delivery of the baby, immediately after the delivery of the placenta

*From The Department of Obstetrics and Gynecology and the Department of Surgery University of Mississippi Medical Center Aided indirectly by Army Contract No DA 49 007 MD 627

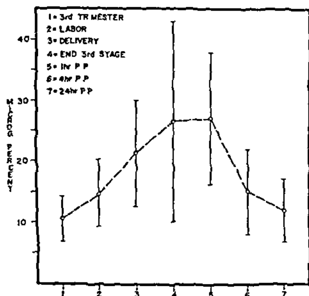


Fig 1 Plasma values of 17 21 hydroxycorticosteroids during labor and after delivery

and 1, 4, and 24 hours postpartum Heparinized syringes were used and the plasma was immediately separated and frozen Determination of free and glucuronide conjugates of 17 21 hydroxycorticosteroids in the plasma were made by the methods of Nelson and Samuels[5] and Bongiovanni[6]

RESULTS

Maternal Data The data are presented graphically in Figure 1 At the 36th week of pregnancy the mean plasma value of 17 21 hydroxycorticosteroids was 10 63 ± 3 83 mcg /100 ml This is somewhat higher than the value of 7 0 mcg /100 ml found by the same laboratory for normal non pregnant women of the same age group During active labor there was a rise to a mean value of 14 71 ± 5 35 mcg /100 ml A further increase to a mean of 21 16 ± 8 58 mcg /100 ml occurred at the time of delivery The differences in mean values between late pregnancy and labor and between labor and delivery are highly significant (p less than 0 01) Following delivery of the placenta, the 17 21 hydroxycorticosteroids rose again to a mean value of 26 50 ± 16 4 mcg /100 ml The peak occurred at 1 hour following delivery At that time the mean plasma value was 27 01 ± 10 80 mcg /100 ml This was not significantly different from that found immediately after delivery of the placenta At 4 and 24 hours postpartum the mean values had decreased to 15 05 ± 7 20 and 11 04 ± 5 24 mcg /100 ml respectively Both these are significantly less than that found 1 hour postpartum and from each other There was no significant difference between the late pregnancy and the 24 hour postpartum mean values

Individual patients showed considerable variation from the mean values as indicated by the standard deviations However, in all cases the values during active labor were higher than in late pregnancy, *in 13 out of 15* the value at delivery of the baby was higher than that during active labor, in 11 out of 15 the value at delivery of the placenta was higher than that at delivery of the baby Changes in values between delivery of the placenta and 1 hour postpartum were inconstant Subsequent decreases were consistent *In 14 cases the value was lower at 4 hours postpartum than 1 hour*

postpartum and in all cases the value at 24 hours was lower than that 1 hours postpartum In 9 patients the 24 hour postpartum value was lower than that found in late pregnancy

Fetal Data Determinations of 17 21 hydroxycorticosteroids were also made on plasma obtained from umbilical cord blood The mean value was 3 37 ± 1 41 mcg /100 ml Although these values are not as high as some previously reported, it is interesting to note that in every instance the maternal level was higher than that in the cord

DISCUSSION

Under normal circumstances 17 21 hydroxycorticosteroids appear to be produced by the adrenal cortex Since adrenal glands examined soon after delivery weigh more and have a larger cortex than those of non pregnant women, it is reasonable to suppose that the maternal adrenal cortex is responsible for the increase in production found during pregnancy and in labor and delivery Activity of the fetal adrenal cortex seems to play no part since the amount of 17 21 hydroxycorticosteroids in cord blood immediately after delivery is about 15% of that in maternal blood However, the placenta has considerable endocrine function during pregnancy and its contribution to the production of these steroids is uncertain

Production of 17 21 hydroxycorticosteroids is probably continuous but may be greatly increased when the body is subjected to stress For example, Hardy and Turner[7] have obtained values of 40 7 mcg /100 ml during and 35 1 mcg /100 ml 4 hours after major surgical procedures The increase over the normal value found in this study at the 36th week of pregnancy is relatively small, and the increases found during and immediately after labor and delivery are not as high and do not persist as long as those observed for major surgical procedures The changes are definite enough, however, to support the belief that pregnancy is a normal physiological stress situation, and that this stress is considerably increased during labor and delivery It should be noted that these patients were all normal and had quite easy labors and deliveries without complications It is possible that alterations in management and abnormalities such as prolonged labor might change the steroid response

SUMMARY AND CONCLUSIONS

1 Serial determinations of plasma 17 21 hydroxycorticosteroids have been made in 15 normal women from the 36th week of pregnancy until 24 hours postpartum

2 Plasma values of 17 21 hydroxycorticosteroids were higher at the end of pregnancy than in normal nonpregnant women A significant rise was observed during labor reaching a maximum 1 hour postpartum By 24 hours postpartum there was a fall to less than that of late pregnancy

3 Values of 17 21 hydroxycorticosteroids were in all instances lower in umbilical cord plasma than in maternal plasma taken immediately after delivery of the baby

4 These findings support the belief that a characteristic response to stress occurs in pregnancy labor and delivery

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THE IMMEDIATE POSTPARTUM CERVIX*

A Colposcopic Study

WARREN R LANG AND PAUL D ZIMSKIND

The immediate postpartum cervix has received but scant attention in the obstetric literature There are a few available references concerning its gross appearance biopsy findings and the accompanying vaginal cytology We have been particularly interested in the immediate postpartum cervix because of the possible relationship of the observed changes to benign cervical erosion The colposcopic technique (stereoscopic visualization *of the cervical portio under magnification with bright illumination*) has been used in our investigations

METHOD

Our case material consists of 81 recently delivered women from the obstetric wards of the Jefferson Medical College Hospital Their ages ranged from 16 to 10 years of age Seventy one of the 81 women were colored the remainder were white Nineteen were primiparas (having just delivered the first child vaginally) 62 were multiparas (having just delivered the second or later child vaginally) Of the cephalic presentations 73 were spontaneous 4 were delivered by low forceps Four cases were delivered by breech extraction after the buttocks had been extruded spontaneously Seven of the cephalic presentations and one of the breech presentations were premature (babies weighing less than 2500 gm) There was one set of twins and one set of triplets The time of colposcopic examination after delivery varied from 13 hours to 103 hours The median time was 10 hours postpartum

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With the aid and advice of Hyman Menduke Ph D Assistant Professor of Biostatistics

Colposcopic examinations were carried out by the usual technique at 10X magnification a Moller colposcope was used[1] A clean lubricated speculum was inserted gently The colposcope was then adjusted into position and the cervical portio carefully inspected Aqueous 3% acetic acid was applied freely to bring out detail Occasionally Lugol s solution (Liquor iodii compositus USP) was used but not routinely Careful notes and diagrams were made of all cases especially of the relative extent and location of columnar epithelium Photographs were taken by the Leica camera attachment of the colposcope Colposcopic examination of the immediate postpartum cervix was found to be somewhat more difficult than that of the nonpregnant or prenatal cervix because of the tenderness of the vagina and perineum following delivery

RESULTS

Colposcopic Findings Unrelated to Recent Delivery There were certain colposcopic findings which were not the result of the delivery just past but which were undoubtedly present beforehand These findings related to types of epithelia (squamous or columnar) and their pattern on the portio In only 3 cases was the portio completely covered by squamous epithelium (multiparas ages 19 20 34) In 13 instances there was a more or less regular rim of columnar epithelium (ectopy) sharply demarcated from squamous epithelium surrounding the anatomical external cervical os (4 primiparas ages 16 to 25 9 multiparas ages 17 to 28) In the remaining 65 cases there were evidences of a transformation zone (i e islands of columnar in squamous epithelium gland openings in squamous epithelium and/or nabothian cysts) with or without ectopy Fourteen of these were primiparas (ages 16 to 29 with a mean age of 20 3 years 51 were multiparas) ages 16 to 40 with a mean age of 27 4 years

Colposcopic Changes From Delivery Itself There were 3 findings the direct result of delivery and these were surprisingly minimal in extent true erosion (loss of epithelium i e an ulcer) laceration and a bruising (ecchymosis) of the anterior cervical lip True erosion occurred in 45 of the 81 cervices examined (55 6%) In 14 of the 45 cases there were 2 areas of true erosion one cervix had 3 such areas and another had 4 The areas of true erosion were usually only 2 or 3 mm in actual diameter and seemed to favor the anterior lip Six cases of true erosion were followed until healing and in each case squamous epithelium grew in Lacerations of the cervix were noted in 25 cases The lacerations seemed to favor no particular o clock and were characteristically no more than 3 to 4 mm in actual length A marked bruised appearance with extravascular infiltration of blood of the anterior lip was found in 3 instances This was originally reported by Hinselmann some time ago[2]

Miscellaneous Observations In our experience the cervical hypertrophy and edema of pregnancy diminish quite soon after delivery The cervical os also closes fairly rapidly so that at the time of our postpartum examination the external os was only 4 or 5 mm in actual diameter There was usually a moderate quantity of dark blood exuding through the os The examinations did not increase the amount of postpartum bleeding nor were there any postpartum infections attributable to the investigation

DISCUSSION

The demonstration that columnar epithelium is frequently found on the portio and that ectopy (an isolated perioral rim of columnar epithelium) seems to favor younger women is in agreement with the investigations of Ganse[3] and with a previous study on nonpregnant women of childbearing age from our own institution However, to be statistically significant a larger series of cases is necessary From our data no conclusions can be reached concerning the relationship between parity and the occurrence of a transformation zone (i e, islands of columnar epithelium gland openings, and/or nabothian cysts)

The relative lack of damage that the cervical portio suffers from delivery is quite surprising In fact, one may consider the epithelial pattern of the immediate postpartum cervix as practically identical with the prenatal cervix except for the minute evidences of trauma previously mentioned Our work is in accord with the histologic observations of Glass and Rosenthal and Revoltella who found minimal loss of epithelium as a result of fetal expulsion

In addition to its mere academic interest there are certain implications to be drawn from a cervix whose epithelial surface remains fairly intact in spite of delivery The usual sequence of events has been thought to be squamous epithelial denudation then a growth of columnar epithelium from the endocervix (which clinically presents on the portio as an erosion) and finally an ingrowth of squamous epithelium toward the external os From our observations although they are preliminary in nature, this does not seem to be true since the areas of epithelial denudation are quite minimal in extent compared to the size of erosions as noted clinically

SUMMARY

1 The cervices of 81 women in the immediate postpartum period were colposcoped (median time after delivery of 40 hours)

2 The immediate postpartum cervix was found to resemble closely the prenatal cervix, the changes produced by delivery consisting of small lacerations bruising and areas of true erosion measuring only a few square millimeters in diameter

3 The implications of the above with respect to benign cervical 'erosion have been discussed

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EFFECTS OF HYPOPHYSECTOMY ON FSH LEVELS AND VAGINAL CORNIFICATION

HANNA KLAUS

A group of 18 patients have been studied for metastatic breast malignancy All have had surgical or X ray castration and cortisone suppression of the adrenals for periods of 2 days to many months prior to eventual hypophysectomy (Fig 1) This is a report of urinary gonadotrophin assays and vaginal smear cornification counts before and after the final operation As a result of these studies we feel that the vaginal smear is a practical and easy way to evaluate the totality of pituitary surgery

Estrogens are one of the most important steroids to eliminate in hormone sensitive breast cancers whether they are of ovarian or adrenal origin Despite a post gonadectomy rise in FSH vaginal cornification often persists presumably due to compensatory adrenal estrogens After hypophysectomy FSH generally decreases to undetectable levels but there is a 1 to 3 week lag period Vaginal smears when atrophic indicate complete adrenal as well as ovarian inactivation trends toward atrophy are seen usually within a week of hypophysectomy complete atrophy in 1 to 3 weeks This test is simpler faster and cheaper than gonadotropin assays

METHOD

Vaginal smears were obtained with a dry cotton tipped applicator inserted into the posterior fornix and then rolled over a slide If the mucosa was dry the swab was moistened in saline prior to use The slide was fixed and stained according to Papanicolaou s method [1] One hundred consecutive cells were counted and classified as basal intermediate precornified and cornified cells At a later date the slide was recounted if each category varied no more than 10% the count was considered valid

FSH was measured on 24 hour collections of refrigerated urine by the mouse uterine weight method of Klinefelter and Albright [2] The lowest detectable level is 2 5 m*u* Values for a normally menstruating woman range between 10 to 20 m*u* at the menopause the levels rise to 100 to 300 m*u*/24 hours

RESULTS

Eight patients did not have increased levels of FSH even after castration and adrenal suppression with corticoids in the presence of advancing disease while 10 patients showed menopausal levels Ten of the 18 had precornified rather than atrophic smears before hypophysectomy After hypophysectomy it took 1 to 10 weeks for FSH to drop to baseline levels with an average time of 1 to 2 weeks (Fig 1) Vaginal smears showed atrophy mostly within 2 to 3 weeks while 1 patient required 9 months (Fig 2) Growth hormone caused cornification in 1 hypophysectomized patient whose smear had been previously atrophic

*From the Department of Surgery Division of Gynecology Peter Bent Brigham Hospital Boston Mass Supported by Grant #NC FIDB T of the National Institutes of Health

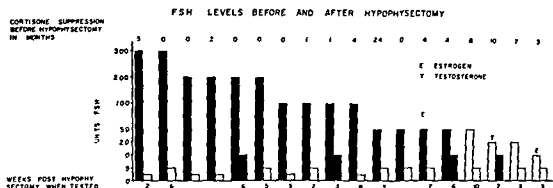


Fig 1 FSH levels before and after hypophysectomy, with or without antecedent cortisone suppression of the adrenals Black columns show highest positive titer white columns show a negative result at the level tested

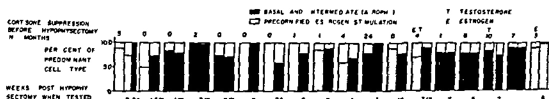


Fig 2 Vaginal smears before and after hypophysectomy with and without antecedent cortisone suppression of the adrenals

DISCUSSION

A complete hypophysectomy deprives the patient of gonadotrophins, adrenocorticotrophins, growth hormone, thyrotrophin, mammogenic hormone, and may cause temporary or permanent diabetes insipidus While testing for ACTH is the most sensitive test for pituitary activity, the need for steroid substitution therapy makes this impractical The thyroid is to some extent autonomous and lack of thyrotrophin is not manifest until 6 weeks post hypophysectomy Aldosterone excretion is not dependent on the pituitary Tests for gonadotrophic activity, while less sensitive than for ACTH, do reflect the completeness of hypophysectomy Many people have studied the relationship of the vaginal smear, FSH and/or urine estrogens after hypophysectomy, notably Lipsett and Pearson[3] who found that the smear showed post menopausal changes 1 week after hypophysectomy and was completely atrophic 3 weeks later At the same time, urinary gonadotrophins usually disappeared 2 to 3 weeks post operatively while some persisted as long as 2 to 3 months in the face of later proven complete hypophysectomy Blackburn and Albert,[4] using Albert's rat ovary method for FSH, found a mean of 5 8 days for complete disappearance after hypophysectomy in 8 cases

Greenwood and Bulbrook[5] studied estrogen of 14 patients after oophorectomy and adrenalectomy in breast cancer patients and found a ⅔ drop in total estrogen output after castration, with no change of the proportions of the 3 estrogens, and only a transient (9 week) drop after adrenal

ectomy The presence of extra adrenals was therefore pointed out and warned against in attempting complete ablative procedures They then similarly studied 10 breast cancer patients who had been hypophysectomized and found 3 patients with no change 4 with a drop in estrogen after hypophysectomy and 3 with a rise The same admonition on completeness of procedures was made

It is interesting that in the absence of ovaries the smears were precornified rather than cornified as was seen first by Dr H Castellanos in our laboratory while ovarian estrogen alone produces a cornified smear This may indicate that the estrogen in our patients is of adrenal origin Since we know that gonadotrophins may elicit estrogen from the adrenal cortex as well as the ovary an atrophic vaginal smear in the presence of intact adrenals can be considered to indicate pituitary ablation As a negative report it should be added that S R changes in the basal cells described by Graham[6] in cervical carcinomas were rarely seen and appear not to be significant in breast cancer

In this series as in Lipsett and Pearson s the correspondence between vaginal atrophy and baseline FSH values has been shown to be good A vaginal smear is an easier cheaper, and faster test than an FSH determination and would appear to be as accurate

SUMMARY AND CONCLUSION

FSH levels and vaginal cornification curves were followed in 18 breast cancer patients before and after hypophysectomy The results were similar, and justify the use of the vaginal smear instead of the longer and more expensive bio assay to test for the totality of hypophysectomy

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SURVIVAL OF OVARIAN HOMOGRAFTS WITHIN MILLIPORE FILTER CHAMBERS IN THE RAT*

HECTOR CASTELLANOS AND SOMERS H STURGIS

The recent development of filters of cellulose esters with graded pore size, has stimulated extensive research in the immune and other mechanisms that cause rejection of tissue homotransplants from one animal to another If transplants are enclosed in a filter chamber with pores so small that host leucocytes are excluded, even if the animal has been previously sensitized by injections of similar tissue, the transplant can survive for periods of months This survival of malignant and benign grafts, demonstrated by Algire and associates,[1] suggested to us the use of similar millipore filter chambers† to study not only the survival but also the persistence of function as well, of endocrine transplants in appropriately deficient hosts

In a previous publication[2] we have recorded evidence of persisting function of ovarian homotransplants in millipore filters in castrated rats up to 60 days after grafting The criteria of function were the persistence of cornified vaginal smears and the dry weight of the uteri at sacrifice compared with the castrate controls It was emphasized that the significance of this lay only in the fact that these filters excluded leucocytes and all other cellular elements, and that it was thus shown that the grafts were capable of responding to gonadotropic stimulation with estrogen production, even though they remained unvascularized We now wish to present a few details of techique and show samples of the grafted tissue that appear to substantiate the probability that the production of estrogen came from the tissues grafted rather than from some other source

Figure 1 shows the preparation of tiny fragments of rat ovary in a lucite ring to which is glued a millipore filter Perhaps from 10 to 20 such fragments, totalling only about 20 30 mg can be enclosed by the addition of a second lucite ring and filter fitting snugly inside the first and sealed with lucite and acetone glue

Figure 2 shows the size of these lucite chambers, and Figure 3 shows a number embedded in the subcutaneous tissues of a rat There is little reaction if sepsis is avoided

In Figure 4 we see the result of castration in a 200 gm control rat reflected by only occasional cornified peaks in almost daily vaginal smears on a scale graded from negative to plus three The weight of the uterus of these ungrafted castrate controls after 60 days was 20 mgs or less

When about a quarter of an ovary was implanted retroperitoneally, we obtained excellent evidence of function over a period of 60 days by vaginal smears and uterine weight (Fig 5)

Figure 6 shows the appearance of the vaginal smear after castration, and before grafting In contrast excellent cornification is seen two months after the transplants have been embedded (Fig 7)

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†Millipore Filter Corp Watertown Mass

*From the Department of Surgery Division of Gynecology Peter Bent Brigham Hospital Boston Mass Supported by an American Cancer Society Institutional Grant to Harvard University

With the technical assistance of John Rahilly

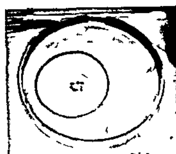


Fig 1 Rat ovary in a lucite ring



Fig 2 Size of lucite chamber

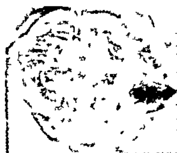


Fig 3 Chambers embedded in subcutaneous tissues of a rat

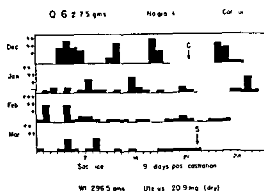


Fig 4 Result of castration in a 200 mg control rat

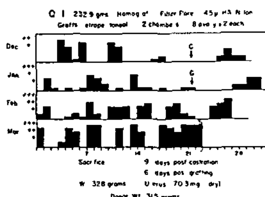


Fig 5 Excellent function of transplant after 60 days



Fig 6 Appearance of vaginal smear after castration and before grafting

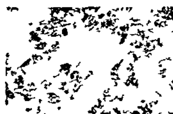


Fig 7 Vaginal smear two months after transplantation



Fig 8 On the right is the uterus of the castrated control, on the left that of a castrated, then grafted animal after 2 months



Fig 9 After 61 days the transplant shows large numbers of clear, round nuclei

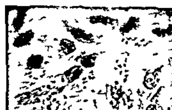


Fig 10 After 96 days there is seen a substrate of fibrocytic cells with an intermixture of larger, round nuclei

The uteri show gross evidence of estrogenic stimulation (Fig 8) On the right is the uterus of the castrated control on the left that of a castrated then grafted animal after 2 months

What then are the cellular elements that seem to survive and function? We have never seen survival of unmistakable follicles and ova after 2 months The tissue removed at sacrifice however is unquestionably healthy and viable After 61 days (Fig 9) there are large numbers of clear round nuclei that may derive from theca or granulosa In a transplant after 96 days there is seen a substrate of vigorous healthy fibrocytic cells with an intermixture of larger round nuclei (Fig 10)

In conclusion we feel we have demonstrated that both survival and function of ovarian fragments can be obtained for at least 90 days when these transplants are embedded in millipore filter chambers in castrated rats even though these chambers exclude vascularization and thus any direct oxygen supply from the general circulation of the host animal These preparations might be considered analagous to tissue cultures *in vivo* Observations on rat ovaries cultured *in vitro*[3] have shown an overgrowth of epithelial structures by fibrocytic elements and degeneration within a few weeks We have seen the same fibrocytic proliferation but nevertheless evidence of persisting estrogen production for more than 3 months in these *in vivo* cultures

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THE CHANGES IN THE SERUM PROTEINS AND LIPOPROTEINS OF PATIENTS IN REMISSION FROM OVARIAN CARCINOMA DURING TREATMENT WITH THIOTEPA*

BERNARD J MILLER DAVID M FARELL AND JOHN C KISTENMACHER

Changes have been noted in the plasma protein patterns of patients with progressive malignant disease[1] There is no evidence as yet to indicate that the malignant state is associated with the sudden appearance of a unique protein or that the disease is associated with any characteristic change in the concentration of these plasma constituents In general

*From the Daniel Baugh Institute of Anatomy The Jefferson Medical College the Department of Gynecology of The Jefferson Medical College and the Department of Surgery of The Germantown Hospital Aided by a grant from Mr M Levine

With the assistance of Dr T L Montgomery and Dr J B Montgomery in providing patients for this study

progressive neoplastic disease is associated with hyperglobulinemia, hypoalbuminemia and hyperfibrinogenemia. Recently, changes have been described in the serum alpha lipoprotein patterns.[2, 3] These molecules decline markedly during the progress of the disease and in some instances are detectable in only trace amounts. The beta lipoprotein concentration rises. One constituent of the beta lipoprotein is thought to be characteristic of metastasis[2]

In a previous communication the serum protein patterns were shown to revert to normal in instances of prolonged remission. The alpha lipoprotein pattern, on the other hand, failed to become normal[3] and were present in excessive quantities in the blood serum during prolonged remissions.

These facts prompted an investigation of the usefulness of serial measurements of the serum protein constituents as a means for rapidly assessing changes in the rate of progression of the disease, or as a means of indicating sustained remission. Accordingly, the serum proteins and lipoproteins were studied by the method of serum electrophoresis in a group of patients with disseminated ovarian malignancy, during treatment with a polyfunctioning alkylating agent.

METHOD

Because of the reports of significant palliation of ovarian carcinoma in some patients treated with N-N'-N" triethylene thiophosphoramide,[1, 5] this drug (Thiotepa, Lederle) was used in a group of 23 patients with advanced inoperable ovarian carcinoma. They ranged in age from 36 to 72 years. In all cases the diagnosis was established by histologic examination of tissues removed at laparotomy by biopsy or by resection of the tumor. All patients were classified as inoperable. With the exception of one case in which pulmonary metastases were demonstrable, the disease was confined to the abdomen in the remaining cases. Triethylene thiophosphoramide was prepared in a solution of 1 mg./cc. of saline. Sterilization of the solution was accomplished by filtration through a Sitz filter and the solution stored at 4°C. The initial course of treatment consisted in the intravenous administration of 50 mg. of the drug given over a period of 7 to 10 days. Generally the initial dose was 10 mg. Thereafter 1 mg. of the drug was given for each 1000 white blood cells until the initial course had been completed.[5] Daily white blood cell counts and platelet counts were performed. Treatment was discontinued if the white blood cell count was depressed to 4000 or if the platelet count was depressed to 150,000/cc. After the initial course of treatment, patients were maintained with an average dose of 15 mg. intravenously every 3 to 4 weeks. When relapse was noted an additional 50 mg. were given in divided doses, provided this did not result in bone marrow depression. Some patients with effusions were treated by the direct intercavitary instillation of the drug.

The serum proteins and the serum lipoproteins were measured by the method of filter paper electrophoresis. Serum was obtained by centrifuging 5 cc. of clotted blood previously obtained by venipuncture. Electrophoresis of the serum was then carried out in a modified Grassman apparatus containing a veronal buffer (pH 8.6, ionic strength .03). Separation was carried out for 12 hours usually at 15°C., using a current of 4×10^{-3}

amperes/cm When dry, each strip was divided longitudinally One of the resulting strips was stained for proteins with brom phenol blue, the other was stained for lipoproteins with sudan black Quantitative densimetric measurements were made by continuous optical scanning The strips stained for proteins were scanned at 6 20 mu while those stained for lipoproteins were scanned at 5 60 mu

OBSERVATIONS

Results of Therapy. Of 23 patients with advanced disseminated ovarian carcinoma, 2 died before the first course of therapy could be concluded Three of the remaining 21 patients failed to exhibit any evidence of improvement during treatment and continued to progress rapidly and finally died The remaining patients showed varying degrees of improvement In 7 instances the reformation of ascites was completely prevented for periods ranging from 3 to 8 months Marked evidence of tumor regression and in some instances disappearance of tumor were observed for periods varying from 3 to 14 months Of the entire series of patients, one who at the time therapy was started showed evidence of rapid reformation of intra abdominal tumors, is apparently still in remission 14 months after therapy was begun Evidence of escape from control was usually manifest by the reappearance of ascites or abdominal masses It was occasionally possible to regain some measure of control by administration of larger quantities of triethylene thiophosphoramide Usually repeated remissions induced by increased dosage of Thiotepa were much more difficult to obtain than the first and in many instances a second or third remission could not be obtained

Changes in Serum Constituents during Therapy with Thiotepa. In 10 cases *the changes in the serum proteins and lipoproteins* were serially followed At the beginning of therapy the serum of all patients showed remarkedly diminished proportions of albumin and alpha lipoproteins (Figs 1, 2) The proportions of gamma globulin appeared to be related to the general condition of the patient Advanced cases, as yet in good general condition,

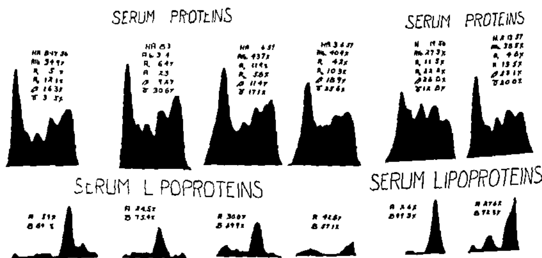


Fig 1 Fig 2

usually presented high values of gamma globulin (Fig 2) The serum of pre terminal or terminal patients, on the other hand, contained markedly reduced proportions of gamma globulin (Fig 2) The alpha globulins particularly the alpha' fragment, were very markedly increased especially in terminal cases

Effectiveness of therapy was usually reflected in an elevation in the proportion of albumin and a decline in the alpha globulins of the serum Frequently the levels of gamma globulin increased momentarily and then subsided In instances in which the levels of gamma globulin were originally low, the rise was less abrupt Clinical remissions were usually associated with near normal levels for the plasma proteins Some patients in remission showed a moderate and sustained elevation in gamma globulin levels

The lipoproteins showed greater changes during remission than did the proteins Marked increases in alpha lipoproteins were nearly always associated with rapid improvement The abnormal beta lipoprotein associated with progression' rapidly diminished in remission The greatest elevation in alpha lipoprotein was noted in one patient, still in remission for 11 months

With relapse, a gradual reversal in both the serum protein and lipoprotein patterns were observed This nearly always preceded clinical evidence of relapse Frequently the inability to maintain a long remission could be predicted by the appearance of only slight changes in the patterns of the serum constituents despite what appeared to be a fair or good clinical response

The changes in the protein and lipoprotein pattern in the serum of a patient during the first 7 months of remission are shown in Figure 1 This patient had massive recurrence of her ovarian carcinoma prior to treatment and is still in remission 11 months after the beginning of therapy Similar changes in the serum of another patient with ascites bilateral pleural effusion and recurrent tumor are shown in Figure 2 This patient relapsed after a remission of 10 months

CONCLUSION

It is beyond the scope of this paper to speculate on the possible existence or absence of tumor antagonistic factors within the host The rapid decline of alpha lipoprotein and gamma globulin in pre terminal patients and the elevation of these constituents in greater than normal amounts with remission may be related to this phenomena

From a clinical point of view the changes in the serum constituents appear to precede changes in clinical status In this regard, the method of serial electrophoresis may assist in the early detection of favorable or unfavorable effects of hormones or cytotoxic agents in the trreatment of disseminated cancer

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THE USE OF FREEZE DRIED HOMOLOGOUS DURA MATER IN NEUROSURGERY*

W EUGENE STERN

The frequent need to repair dural defects has led us to apply to human dura mater the principles of arterial graft preservation stated by Hufnagel, Rabil, and Reed [1]

Since February, 1956 specimens of fresh human autopsy dura mater have been prepared for anticipated neurosurgical needs The tissue is taken at routine autopsy examination under unsterile conditions and placed in a saline solution to which is added both aqueous penicillin and streptomycin After trimming the desired shapes and sizes, the tissues are sterilized by immersion in ethylene oxide for 30 minutes Quick freezing is accomplished in 10 minutes with carbon dioxide ice and 95% ethyl alcohol, following which the specimens may be stored (at −70°C) until sufficient numbers are available to justify operating the freeze-dryer The final step is made by removing all moisture in a vacuum of 0 3 to 0 4 microns for 24 hours The dura is then sealed in glass tubes ready for storage Room temperature storage of the "banked" dura mater has been our custom The tissue is reconstituted at the operating table by removing it from the sealed container under sterile precautions and immersing it in physiologic salt solution or distilled water for 20 to 30 minutes In this state it is ready for use It is tough, pliable, and easily handled — a bit more chalky in color and a bit more leathery or "chamois like", but otherwise physically similar to normal dura mater in its surgical properties

METHOD

Fifteen patients have received dural grafts, beginning with the first case on April 5, 1956, two months after the preparation of the first specimen for storage The graft for this first patient was implanted for repair of a dural defect produced by the total excision of a parasagittal, psammomatous meningioma

The grafts have varied in size from 16 to 96 sq cm The largest was implanted to replace excised dura mater which was involved by a chordoma overlying the midline posterior fossa structures extending from the foramen magnum to the torcular Herophili

No reactions have been witnessed that might be attributed to the grafts, although the first patient still has focal seizures from her meningioma site These attacks had been present preoperatively for 25 hours, and since their incidence and nature have not changed (except to improve), they do

*From the Department of Surgery University of California Medical Center, Los Angeles With the consultative services of Drs Clarence Johnston and W Jann Brown Department of Pathology and Dr Jack Cannon, Department of Surgery

not seem to incriminate the graft implantation No wound infections have occurred No wound drainage has occurred No graft has had to be removed or replaced Cultures of the final product at the time of operation are available in 6 cases, 5 were sterile and the 6th grew a coagulase negative micrococcus, although no trouble occurred within the patients wound

The grafts have lent themselves to various uses (1) replacement of dura mater excised for meningiomas 6 cases, (2) replacement of dura mater over orbital roof 1 case, (3) adjunct to decompressive procedures 2 cases (1 temporal decompression, and 1 posterior fossa decompression) (4) miscellaneous intracranial needs 4 cases, (5) extracranial applications, 2 cases (1 decompressive laminectomy, and 1 repair of chest wall)

DISCUSSION

Histologic study of the experimental animal dural graft preparations by Sewell and associates (1954 to 1955)[2,3] demonstrated that invading fibroblasts replace the cells of the graft to lay down new collagen Some slight adhesions were noted between their experimental grafts and the pia arachnoid No change in properties occurred, and they observed no shrinkage of the graft

Comparison of histologic preparation of fresh autopsy dura mater and preserved dura mater from the same autopsy specimen demonstrates a lack of cellularity and a tendency for the collagen layers of the graft tissue to separate

Autopsy material secured on the 8th postoperative day from the patient in Case 12 revealed no adhesive reaction between graft and host brain Histologic study demonstrated a distinct line of separation between the two tissue segments, acellularity of the donor tissue, and a mild cellular reaction on the under (brain) surface of the suture line Erythrocytes could be seen, however, within channels of the graft tissue A second stage procedure performed on the patient in Case 5, 4 months following implantation, permitted examination of the reaction of the graft and host tissue The dural graft was more pale and ivory colored than the host dura mater The excess dural graft tissue, which had been implanted to permit brain expansion if needed, had formed a firm fold at its attachment to the host tissue The latter had calcified and ossified, whereas the graft tissue had not

Histologic examination of the suture line, made in consultation with Dr Clarence Johnson, may be described as follows A syncytium of thick trabeculated, intradural bone is noted in the host dura and remnants of the silk suture material can be identified The graft dura is vascularized with sparsely placed capillaries containing blood and appears viable There is an incomplete break in continuity at the junction of host and graft dura On the inner aspect (pial side) of both host and graft dura is a coagulum mixed with fresh blood blending with structures of a more fibrillar nature, possibly in part representing degenerated collagen of the inner layer of the host and graft dura mater The outer aspect of the host and graft dura is covered with a separate layer of relatively acellular collagenous tissue containing small blood vessels and capillaries On the

host side this layer is separated from the dura mater by bundles of striated muscle (temporalis muscle)

The homologous graft tissue described is superior to known synthetic products and autogenous grafts, since it covers defects with a membrane whose properties approximate those of the host tissue more closely than any other known. The graft need only serve a mechanical purpose; there is no need for metabolic activity or survival of the graft cells. As a consequence, by their physical properties, these banked sheets of dura mater are admirably suited for the needs described Invasion by host mesodermal tissue and permanent replacement by the host of the graft skeleton seem to be the ultimate fate of these membranes. Whether the center of the largest grafts can be vascularized before necrosis occurs is as yet unanswered.

SUMMARY AND CONCLUSIONS

Sterilized and freeze-dried homologous dura mater has been utilized since April, 1956 in 15 cases requiring repair of dura mater or fascial defects. It is admirably suited to storage in sealed ampules at room temperature and is readily available for surgical application after brief reconstitution in water or saline solution at the operating table. No untoward reactions have attended its use Watertight, tough, pliable dural replacements for defect as large as 96 sq cm have successfully been accomplished. This form of graft material appears superior to any other dural replacement, synthetic or otherwise, currently available

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BLOOD LOSS IN INTRACRANIAL OPERATIONS AS DETERMINED BY RADIOACTIVE CHROMIUM[51] TAGGED RED BLOOD CELLS AND IODINATED HUMAN SERUM ALBUMIN*

EDMUND A SMOLIK AND FRANCIS P NASH

The availability of radioisotopes for the accurate determination of blood volume[1] suggested that a re investigation of the problem of blood loss experienced in intracranial surgery would be worthwhile

*From the Department of Surgery (Section of Neurosurgery), St Louis University School of Medicine Performed under A E C Contract AT (11 1) 215

Recent reports by Stanton and associates[2] on the measurement of blood loss during thoracic and abdominal operations have demonstrated that the amount of blood removed from the wound at the time of operation may not represent the actual amount of blood lost to the circulation. These studies have shown that in general surgical patients there is a further blood loss which occurs following the surgical procedure. This postoperative blood depletion is principally of the cellular elements of the blood and is often greater in volume than the amount of blood lost during the operation itself.

Previous studies of the blood loss in intracranial operations have been limited to the measurement of the blood removed from the wound at the time of surgery. We wish to report our studies in which radioactive isotopes were used to determine both (1) the operative and (2) the postoperative blood loss in a series of patients undergoing craniotomy.

METHOD

Radioactive iodinated human serum albumin or Cr^{51} tagged red blood cells were used to measure blood volume in 32 neurosurgical patients. Twenty-nine patients in this series were operated for removal of brain tumor. Two patients had intracranial sections of cranial nerves and one patient was operated upon for the removal of an intracerebellar hematoma.

We used I^{131}HSA in 24 patients to measure the whole blood volume. This technique also directly measured the plasma volume changes. In 8 *patients we selected radioactive* Cr^{51} *tagged red blood cells to determine* whole blood volume. Employing this method we were also able to directly measure the red cell volume changes. Thus in comparable patients either the plasma volume or the red cell volume was directly measured in the process of whole blood volume determination.

In each instance these volumetric determinations were made on the day preceding surgery, immediately after operation and daily for a variable period depending upon the patient's clinical condition.

RESULTS

In 28 patients in this series (87%) there was *in addition to the operative* blood loss a secondary blood loss which occurred in the days immediately following the surgical operation. This blood depletion usually reached its maximum about the third day following surgery.

This postoperative blood loss is principally of the cellular elements of the blood and was sometimes greater in volume than the amount of blood lost during the surgical procedure.

In each instance concurrent with the loss of red cells there was an increase in the patient's plasma volume.

These two alterations in the cell and plasma volumes combined to *produce a marked postoperative anemia.*

Figures 1, 2 and 3 are illustrative of the blood volumetric changes observed in this series of patients.

The average total whole blood loss per patient was 1028 ccm. The total red cell volume loss per patient averaged 615 ccm. Based on the average hematocrit values (45 vol. %) this volume of cell loss would

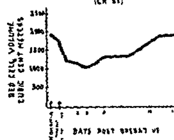


Fig 1 Note the drop in red cell volume by the third postoperative day to almost one half the immediate postoperative volume

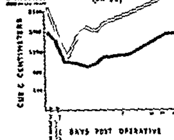


Fig 2 Note the changes in the plasma volume accompanying the postoperative drop in red cell volume

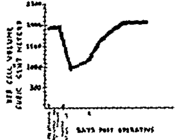


Fig 3 Note the acute drop in red cell volume in the first 24 hour period following surgery

represent a red cell volume supplied by 1366 ccm of whole blood This averages 338 ccm greater than the average measured whole blood volume loss of 1028 ccm

DISCUSSION

Our data indicates that the "total" surgical blood loss in patients undergoing intracranial surgery is in part the blood lost at operation and in part the subsequent loss during physiologic adjustment to the procedure itself

This secondary blood loss is usually insidious and not accompanied by the usual clinical signs of hemorrhage In 28 of the 32 patients in this series the hemoglobin and hematocrit values dropped in the first few days following surgery These drops paralleled in intensity the degree of postoperative decrease of red cell volume and of plasma volume increase These alterations usually reached a maximum about the third day following surgery

These studies do not indicate the mechanism of this postoperative blood cell loss Of importance, however, is that these studies point out the profound changes that may occur in the blood vascular compartments following intracranial surgery Their correction to normal levels establishes normal circulatory dynamics and oxygenation In many instances this is best attained by replacement transfusions of whole blood Clinical improvement of the patient often parallels the adjustment of the blood volume and composition to normal levels

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CEREBRAL BLOOD FLOW DURING EXTRACORPOREAL CIRCULATION*

OSCAR CREECH, JR, EMANUEL BRESLER, MAX HALLEY, AND MAURICE ADAM

While extracorporeal circulation has been firmly established as a useful tool in the treatment of certain cardiovascular conditions, many of the physiologic changes developing during its use are poorly understood It has been demonstrated that low perfusion rates (35 cc/kg/min) are tolerated for relatively short periods of time, however, it is becoming apparent that flows approaching normal cardiac output are more satisfactory [1]

Blood flow of individual organs under the conditions of extracorporeal circulation has received little attention Beall and associates[2] demonstrated a renal blood flow of only 25% of control values during cardiopulmonary bypass with a perfusion rate of 35 cc/kg/min This is a reduction in renal blood flow of the same magnitude as that occurring in systemic flow Recently 3 patients who were operated upon under extracorporeal circulation for atrial or ventricular septal defects developed central nervous system changes after operation that were indicative of cerebral damage Since the clinical manifestations suggested cerebral anoxia, although perfusion rates were at or above basal flows and arterial oxygen saturation near 100%, it occurred to us that a selective reduction in cerebral blood flow might have been responsible for the neurologic disturbances This report is concerned with some experiments designed to measure cerebral blood flow during extracorporeal circulation at minimal and maximal flow rates

METHOD

The method of measuring cerebral bood flow was modified from that described by Rosomoff and Holaday [3] This technique, employing a magnetic rotameter, was selected because it allowed continuous observation of cerebral flow

Mongrel dogs averaging 14 kg in weight were anesthetized with nembutal administered intravenously in a dose of 15 mg/kg Through a midline cervical incision the common carotid arteries were mobilized and all branches except the internal carotid artery ligated An endotracheal tube was inserted and connected to a mechanical respirator set to deliver room air at a rate of 16 cycles per minute The sternum was split vertically and a rib spreader inserted exposing the heart and great vessels Following heparinization a cannula was inserted into the left femoral artery and connected to a Statham strain gauge for measurement of blood pressure The vertebral arteries were ligated at their origin Catheters were placed proximally in the common carotid arteries which directed blood flow from the aorta to a continuously recording magnetic rotameter [4] Similar catheters in the distal carotid and vertebral arteries directed blood from the rotameter to the brain (Fig 1) A small catheter was placed into the

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Supported by grant #HTS 5170 National Heart Institute Public Health Service

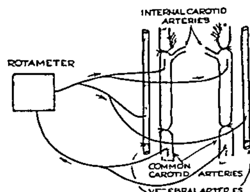


Fig 1 Diagram of arterial circulation and direction of flow through the rotameter for measurement of cerebral blood in the dog

right vertebral vein and directed cephalad for collection of venous blood samples Arterial samples were obtained from the carotid artery Oxygen content was determined by the method of Scholander and Roughton[4] Cerebral blood flow and arterial blood pressure were recorded simultaneously on a direct writing oscillograph

The pericardium was incised and tapes passed about the venae cavae Catheters were placed into the cave through the right atrial appendage and connected to a Sigmamotor pump and bubble oxygenator Oxygenation was achieved with 100% oxygen delivered at a rate of 5 L/min The right femoral artery was cannulated for arterial inflow from the extracorporeal circuit Temperature was measured throughout the experiment by means of a thermistor probe implanted in the subcutaneous tissue A Thermo-Rite® blanket beneath the animal maintained the temperature at about 98°F When blood pressure and cerebral blood flow had stabilized, 2 blood samples were drawn 2 to 3 minutes apart from the carotid artery and vertebral vein Upon completion of control observations, lasting 10 to 15 minutes perfusion was begun In 7 experiments cardiac arrest was produced by the intra aortic administration of 25% potassium citrate In all experiments right atriotomy or ventriculotomy was performed In 6 instances perfusion was begun at a maximum rate of flow and in the remainder it was arbitrarily set at 25 to 35 cc/kg/min When flow was stabilized arterial and venous blood samples were drawn 1 to 3 minutes apart After about 5 minutes, the perfusion rate was increased to a maximum or decreased to a basal level depending upon the initial rate of flow Arterial and venous blood samples were then taken In 3 instances upon completion of this part of the experiment, a mixture of 95% oxygen 5% carbon dioxide was substituted for 100% oxygen in the oxygenator column Observations at maximum and basal flows were repeated, the animals allowed to recover and final control observations then made to complete the experiments (Fig 2) In 4 instances a vasopressor substance (norepinephrine) was administered at the end of the experiment in order to observe the simultaneous effect on arterial blood pressure and cerebral blood flow

At the conclusion of each experiment the brain was removed and its net weight determined

RESULTS

There were 12 experiments performed, 9 of which were technically satisfactory, and these form the basis for this report Perfusion rates

varied from a minimum of 10 to a maximum of 70 cc/kg/min, the average basal flow being about 25 cc/kg/min and the maximum about 50 cc/kg/min

On the basis of the values for blood pressure, cerebral flow, and cerebral oxygen consumption the 9 experiments fall into 2 categories The first consists of 6 experiments in which control observations were satisfactory, i e, the values for blood pressure, cerebral flow, and oxygen consumption were at or near normal levels, and thus constitute valid controls In the second group consisting of three experiments, control observations were made when the animals were hypotensive and cerebral blood flow and oxygen consumption were severely reduced Thus, it is questionable whether subsequent observations during extracorporeal circulation are valid

Cerebral blood flow in the controls ranged from 16 to 60 cc/100 gm/min, the majority of determinations falling within the 30 to 55 cc range Cerebral blood flow during extracorporeal circulation varied from 3 to 73 cc/100 gm/min, a majority of determinations falling within the 15 to 40 cc range Comparison of cerebral blood flow and arterial blood pressure indicates that these 2 factors have a linear relationship, both in the control observations and those made during extracorporeal circulation (Fig 3) Similarly, cerebral blood flow as measured by the rotameter and cerebral oxygen consumption were related (Fig 4) Thus, at low rotameter flows oxygen consumption was reduced while an increase was associated with a similar increase in cerebral oxygen consumption On the other hand, a less definite relationship was apparent between cerebral blood flow and perfusion rate (Fig 5), i e, an increased perfusion rate generally resulted in an increase in cerebral blood flow but to a variable and somewhat unpredictable extent

In all of the experiments a decrease in cerebral oxygen consumption occurred during extracorporeal circulation Excluding the 3 experiments in which control oxygen consumptions were below normal values, a reduction of at least 50% below control values were noted in each of

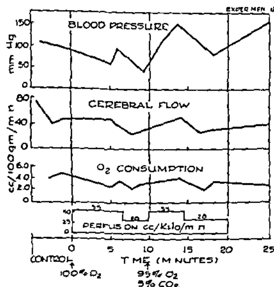


Fig 2 Chronology of an experiment during simultaneous measurement of arterial blood pressure cerebral blood flow and cerebral oxygen consumption

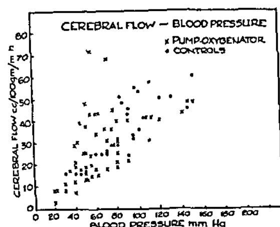


Fig 3 Cerebral blood flow increases in a linear fashion with increase in arterial blood pressure

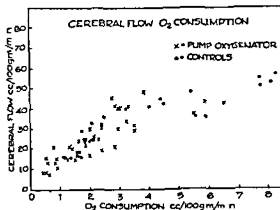


Fig 4 Cerebral oxygen consumption and blood flow show a linear relationship except at high levels

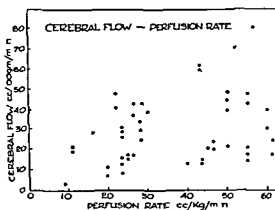


Fig 5 Cerebral blood flow varies widely with changes in perfusion rate

the remaining six experiments In general, this was a result of narrowing of the arterial venous oxygen difference due largely to an increase in venous oxygen content This demonstrates that the rate of oxygen consumption was not flow limited The defect therefore would have to be explained in one of three ways (1) uneven regional distribution of cerebral blood flow, (2) impaired diffusion of oxygen to tissue, (3) impaired utilization of oxygen by tissue, or a combination of these

The addition of 5% carbon dioxide to the venous blood in the oxygenator produced equivocal results in 3 experiments In 2 there was a slight increase in cerebral blood flow at maximum perfusion rates with no significant change at low flows whereas in the third experiment cerebral flow was actually reduced during administration of carbon dioxide

Administration of norepinephrine at the termination of 2 of the experiments clearly demonstrated the approximate linear relationship between cerebral blood flow and arterial blood pressure

CONCLUSION

It should be pointed out that the technique employed in these experiments does not completely isolate the cerebral circulation because of

anastomotic channels about the eye It is believed however, that the major portion of flow through the rotameter was distributed to the brain and thus the changes observed are representative of corresponding alterations of cerebral blood flow

The observations made during this study are too limited to be conclusive The data suggest however that cerebral blood flow during extracorporeal circulation is more dependent upon arterial blood pressure than perfusion rate It would appear that maximum perfusion rates are desirable in order to maintain the blood pressure at as near normal levels as possible

The decrease in oxygen consumption during cardiopulmonary bypass in this series of experiments is an interesting observation the significance of which is obscure However the nature of the preparation precluded complete recovery of the animal at the termination of the experiments Thus it is possible that some may have sustained cerebral damage during perfusion which was responsible for alterations in cerebral oxygen consumption

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NEUROSURGICAL LESIONS FOUND IN A PILOT STUDY OF STROKE PATIENTS*

D W LINDNER J E WEBSTER AND E S GURDJIAN

The surgeon s role in the management of patients with vascular brain lesions has been until recently one of demonstrating and relieving mainly subdural and epidural hematomas Since the thirties intracranial aneurysms and their complications have received widespread attention More recently an increasing number of patients bearing the diagnosis of stroke or cerebrovascular accident have been referred to the neurosurgeon for evaluation

In a recent series of stroke patients studied by cerebral angiography it has been of interest to observe the variety of lesions encountered Of the first 175 patients studied the following lesions have been found

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I *Occlusive Lesions*	
Carotid artery (complete)	19 (3 bilat.)
Carotid artery (partial)	17
Anterior cerebral	14
Middle cerebral trunk	9
Basilar artery (partial)	13
Basilar artery (complete)	4
Embolism	10
Arteriolar disease	27
II *Surgical Lesions*	
Brain tumor	15
Intracerebral hematoma	4
Subdural hematoma	3
Brain abscess	1
III *Others*	
Cerebral hemorrhage (infarction)	7
Cerebrovascular insufficiency	7
Saccular aneurysm	3
Post-cerebral artery occlusion	2
Cerebral atrophy	2
Post ictal state	1
Syphilis	1
Encephalitis	1
Miscellaneous	15

It is, of course, important first to diagnose and treat accordingly the 10% of misdiagnosed patients who do not have cerebrovascular disease, but mass lesions instead. Of the 15 brain tumors encountered, 5 were meningiomas, 2 were brainstem tumors, 4 were astrocytomas, and 4 were metastatic tumors.

Patients with complete occlusion of one internal carotid artery presented symptoms and signs which ran the neurologic gamut from dizziness to total hemiplegia. These patients have been treated by surgical excision of a part of the involved artery and cervical sympathectomy. Any measure which increases collateral circulation through the external carotid system would seem to be worthwhile.

Patients with partially occluding lesions in the carotid arteries commonly have bouts of cerebrovascular insufficiency. These transient episodes have often been referred to as "spasms" of cerebral vessels, or "little strokes." In our experience, the mechanisms of spasm in the cerebral vessels cannot be validated. Consideration must also be given to emboli, the source of which is the ulcerating plaques at the carotid bulb, producing more distal occlusions.

Those patients with segmental atheromatous involvement of the carotid bulb and the internal and external carotids at their origins have been treated by an intimectomy with removal of the plaques and stenotic areas of the lumina. These patients have been previously treated by carotid compression to induce collateral flow, and by anticoagulant therapy.

A small group of patients with cerebrovascular disease as their primary diagnosis fall into the group of "mass" lesions These are the patients with massive hemorrhagic infarction They have been treated by decompression and aspiration, or removal of the intracerebral clot

SUMMARY

A multiplicity of lesions described as "strokes have been found in a study of 175 patients admitted to a cerebrovascular ward for purposes of complete neurological evaluation employing angiographic studies More than 10% of the patients had mass lesions Occlusive carotid artery disease was strikingly common as a cause for cerebrovascular insufficiency and failure

The treatment of the patient with a "stroke syndrome concerns first dealing with the mass intracranial lesions Partial occlusion of the carotid artery has been treated by thromboendarterectomy with success Anti coagulant therapy and therapeutic carotid artery compression may also improve blood flow

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SOME SIMPLE METHODS OF TREATING COMMUNICATING HYDROCEPHALUS*

O HUGH FULCHER AND FRANCIS ENOMOTO

The cerebrospinal fluid represents a lake the source of which is the arterial system and the outlet of which is the venous system If the formation of cerebrospinal fluid exceeds that of absorption there develops hydrocephalus If there exists a block between the ventricles and the subarachnoid spaces the hydrocephalus is called the obstructive type, if the ventricular system is in communication with the subarachnoid spaces the hydrocephalus is termed the communicating type

The methods of making the diagnosis is well described by Matson[1] It is imperative to make an early accurate diagnosis not only to institute therapy for the type of hydrocephalus but to exclude the existence of subdural hematoma or brain tumor Since subdural hematoma is readily amenable to appropriate treatment and since the method of diagnosis is so simple it would indeed be a tragedy to permit an infant to continue to suffer with this condition

It was not until the second decade of the twentieth century that the chief source of the cerebrospinal fluid was demonstrated to be the choroid plexus and the chief means of absorption to occur through the subarachnoid villi Thus in communicating hydrocephalus either the activity of the

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choroid plexus is increased or that of the subarachnoid villi is decreased Consequently the rationale for treating communicating hydrocephalus is evident, either to effect a decrease in the production of cerebrospinal fluid or to drain off the excessive amount The problem appears simple enough but it has not hitherto been solved with any degree of consistency Bach and Walker[2] have given a concise history of the efforts of physicians to treat this condition The story is largely a narration of disappointments and failures

The difficulties of treatment may exist largely because of the sclerosing activity of the cerebrospinal fluid which tends to produce an occlusion of any artificial opening into the subarachnoid spaces

Matson has been largely responsible for the renewed interest in the treatment of communicating hydrocephalus He has obtained excellent results in many instances by employing arachnoid ureterostomy We have used this procedure and have observed that the usually diminished serum chlorides in these patients did disappear occasionally without the recurrence of hydrocephalus This observation has been interpreted as indicating the cessation of drainage of cerebrospinal fluid into the bladder In one or two instances the polyethylene tube has been observed to be occluded Therefore, some patients were able to develop the drainage mechanism when the pressure of the cerebrospinal fluid had been kept adequately reduced

Studies on the newborn have indicated that frequently the infant must develop the absorbing mechanism for cerebrospinal fluid after birth This fluid which exerts an equal pressure in all directions tends to make any enveloping structure become a sphere which appeared to constitute the basis for the rounding out of the baby s head during the first few days of life When the absorbing mechanism failed to develop adequately there developed early signs of hydrocephalus which in some cases disappeared after repeated spinal punctures Thus either the communicating hydrocephalus became arrested spontaneously or else the repeated lowering of the subarachnoid pressure permitted the formation of subarachnoid villi Incidently, we have observed an infant who developed the obstructive type of hydrocephalus while receiving this type of therapy

In 1932 one of us treated communicating hydrocephalus successfully in an infant by introducing a braided silk suture into the subarachnoid space and placing the ends in the adjacent muscle tissue In this manner it was thought that the excess cerebrospinal fluid was drained into the muscle tissue Subsequently this procedure was used several times with occasional success

In 1939 one of us attempted to drain off the cerebrospinal fluid by planting intact muscle tissue into the subarachnoid spaces Much to his surprise it aggravated the condition It appeared that the intact muscle tissue constituted a source of the cerebrospinal fluid rather than an outlet as was desired

In 1946 one of us treated several patients with communicating hydrocephalus by Hiller s method of using the seton. Some of the patients did improve but it was difficult to evaluate the procedure It was not determined whether the improvement had resulted from the repeated spinal punctures or had resulted from the drainage promoted by the setons

About four years ago our attention was directed to the epidural space as a possible site of absorption for the excess cerebrospinal fluid The anesthetists were using epidural anesthesia at that time rather frequently for certain operative procedures and it was observed that the anesthetic agent would frequently be eliminated so rapidly that a continuous administration had to be developed It was also observed that patients who were treated by epidural injection of a weak novocaine solution did frequently develop a reaction presumably because of rapid absorption

Our investigation revealed that the epidural space of an adult could absorb 4000 cc of normal saline during 24 hours Furthermore when phenolsulfonphthalein was placed in the epidural space it was excreted as rapidly during the first two hours as if it had been given intravenously Subsequently we performed various operative procedures on patients suffering with communicating hydrocephalus to drain the excess cerebrospinal fluid into the epidural space In most instances a hemilaminectomy was performed and polyethylene tubes of various forms and shapes were used to promote the drainage In most of these patients the tube would function only for 3 to 4 weeks During this time the pressure of the cerebrospinal fluid varied from 80 mm to 100 mm of water It was observed however that some of these patients did benefit by this procedure as the subsequent course of the hydrocephalus appeared to be favorably modified

An evaluation of our experience at this time seems to indicate that the use of repeated spinal punctures the use of the braided silk suture and the use of the setons had probably not drained the cerebrospinal fluid into the tissues but had actually drained it into the epidural space We have observed at operation that a perforation through the dura and arachnoid made by a previous spinal puncture had continued to leak fluid into the epidural space after seven days Furthermore it occurred to us that the treatment of communicating hydrocephalus perhaps did not have to cover the span of a life time There were indications that if the pressure of the cerebrospinal fluid could be kept reduced for a period of a few weeks to a few months that an adequate mechanism for absorption could develop It was obviously impractical to employ repeatedly an open surgical procedure to promote the drainage into the epidural space Therefore one of us devised a polyethylene tube which could be introduced into its proper position by lumbar puncture performed with a special spinal needle (Fig 1)

A spinal puncture was made between the third and fourth spinous processes with a No 14 gauge Touhy spinal puncture needle after making a little nick in the skin A polyethylene tube size 3 8 F was bent at right angles about 2 cm from the end and some small perforations were made in the distal portion A window was made in this tube just proximal to the right angle and a small piece of the polyethylene wall was elevated by heating in hot water This semi-open window was designed to buttress against the dura to maintain the correct position of the tube and to assure that the cerebrospinal fluid was drained into the epidural space The correctly marked polyethylene tube was then threaded through the spinal puncture needle the needle was withdrawn the tube was adjusted and was cut beneath the surface of the skin A single silk suture was then used to anchor it to the fascia The nick in the skin was then closed

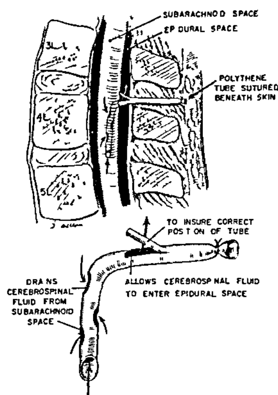


Fig 1 Polyethylene tube *in situ* The special spinal needle has been withdrawn

with a single suture When the tube became blocked after 2 to 4 weeks the procedure could be repeated with little trouble This simple procedure appeared to be as effective in draining the cerebrospinal fluid into the epidural space as had been the open operative procedures Subsequently, it was learned that a smaller tube than 3 8 F was desirable Later we learned that the same procedure could be done through the sacral hiatus and that the tube could be equipped with multiple buttresses to prevent its migration into the subarachnoid space After the spinal puncture has been performed with a large needle the intraventricular pressure should always be lowered by a ventricular tap to avoid the development of an obstructive hydrocephalus At the present time no nick is made in the skin After the spinal needle has been withdrawn the polyethylene tube is severed and shoved through the skin and the small perforation is filled with colloidan The circumference of the head of the patient has been measured at frequent intervals Whenever the head has begun to get larger the procedure has been repeated

RESULTS

This simple method of draining the cerebrospinal fluid into the epidural space has been used on 8 patients The age of these patients varied from 8 months to 14 months at the time the treatment was begun Six are alive, 2 have had 3 procedures, 3 have had 2 procedures, and 1 has had 1 Adequate absorbing mechanism appears to have developed in 4 patients

Two patients developed an obstructive hydrocephalus and subsequently died The autopsy revealed that in one case the obstruction had occurred at the foramina of Luschka and that the foramen of Magendie was absent In the other patient the obstruction was due to the collapse of the cerebral

aqueduct In each instance the obstructive hydrocephalus had been precipitated by the use of a large spinal puncture needle permitting drainage to occur without previously reducing the intraventricular pressure by a ventricular tap

While the cerebrospinal fluid was drained adequately into the epidural space, the subarachnoid pressure varied from 80 mm to 100 mm of water which appeared adequate to prevent a collapse of the ventricles

DISCUSSION

An adequate absorbing mechanism appeared to develop in 4 of the patients during a period of 4 to 10 weeks when the pressure of the cerebrospinal fluid had been maintained at 80 mm to 100 mm of water There were 2 failures Perhaps adequate treatment for infants does not have to be planned to be longer than 10 to 12 weeks

It occurred to us that some of the ventriculostomies may have failed *because the cerebrospinal fluid was dumped into the subarachnoid space* when it had not developed subarachnoid villi Some such patients did do well when frequent spinal punctures were performed We have observed that occasionally after a Torkildsen procedure that one has to perform spinal punctures frequently for a few days before the tube has functioned properly

A large polyethylene tube was not desirable It became plugged more quickly than a small one Furthermore, a large tube could drain the fluid faster than it could escape from the ventricles which did occasionally precipitate additional complications In 2 instances a communicating hydrocephalus was converted into an obstructive type

The dilatation of the ventricles was as obvious in communicating hydrocephalus as it was in the obstructive type It was this dilatation that was probably responsible for the brain damage rather than the increased intracranial pressure Since hydrostatic pressure is the same in all directions and since the ventricles communicate with the subarachnoid space in communicating hydrocephalus, one may wonder why they should become dilated It is evident that some additional pressure within the ventricles is required to overcome the opposing force of elasticity of the walls and of the surrounding cerebral tissue We think that the hydrostatic pressure on the midbrain and on the walls of the fourth ventricle does produce partial obstruction Hydrostatic pressure on the midbrain is transmitted to the walls of the cerebral aqueduct and causes it to collapse In this instance a greater pressure within the ventricles is required to force the cerebrospinal fluid through the collapsed cerebral aqueduct

CONCLUSION

The cerebrospinal fluid can be drained into the epidural space by the use of a small polyethylene tube placed properly by subarachnoid puncture with a special spinal needle This subarachnoid puncture may be performed through the sacral hiatus The epidural space has adequate *capacity to absorb the excess cerebrospinal* fluid in patients suffering with communicating hydrocephalus The pressure within the subarachnoid space was maintained at 80 mm to 100 mm of water when the excess fluid

was drained into the epidural space This is thought to be the pressure of absorption by the epidural space When this pressure was maintained during a period of 6 to 10 weeks an adequate absorbing mechanism for the cerebrospinal fluid of the average infant appeared to develop

The cerebrospinal fluid is a mild sclerosing agent which probably accounted for the fact that the polyethylene tubes did not function longer than 3 to 4 weeks

The dilatation of the ventricles was as obvious in communicating hydrocephalus as in obstructive type This increased intraventricular pressure over the subarachnoid pressure has been attributed to a partial obstruction of the cerebral aqueduct or the foramina of Luschka due to hydrostatic force The dilatation of the ventricles and not the increased intracranial pressure appeared to be the chief cause for irreparable damage to the brain Early treatment is mandatory to preserve cerebral function

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EXPERIMENTAL OCCLUSION OF DURAL SINUSES*

GUY OWENS GRAY STAHLMAN JOE M CAPPS AND A M MEIROWSKY

Clinical evaluation of neurologic deficits accompanying dural sinus injuries is complicated by the associated extensive craniocerebral trauma This study was undertaken in order to establish experimentally the vascular and neurologic alterations which are produced by occlusion of the superficial cerebral venous drainage system when unassociated with cerebral damage Similar work[1] has been reported using dogs as the experimental animals In order to decrease the phylogenetic gap between man and dog the Macacus Rhesus has been studied

METHOD

Twenty six adult Macacus Rhesus monkeys were subjected to biparietal or bifrontoparietal craniectomies Changes in the superficial cerebral venous drainage were produced by ligation and by intraluminal insertion of skeletal muscle or paraffin These alterations were demonstrated by sinograms and in special situations roentgenograms of the venous phase of carotid artery injections were attempted Thirty five and 75% diodrast solutions were used as the contrast material

*From the Department of Surgery Vanderbilt University School of Medicine Nashville
Supported by a grant from the U S Army Medical Department Research and Development Division

Unipolar and bipolar electrocorticograms were obtained from the parietal regions of 5 animals before and during sinus occlusion Simultaneous cerebrospinal fluid pressure changes were measured in these 5 animals by connecting a water manometer to a #18 gauge needle inserted into the cisterna magna

Gross and microscopic examinations of specimens of brain and dural sinus were obtained from 16 animals sacrificed from 5 days to 6 months after completion of the experiment All types of occlusion were represented

RESULTS

Venous pathways were obstructed at the following sites rolandic veins bilaterally (3 animals) superior longitudinal sinus tributaries bilaterally (3 animals) superior longitudinal sinus tributaries unilaterally (2 animals) superior longitudinal sinus anterior to rolandic veins (3 animals) superior longitudinal sinus posterior to rolandic veins (16 animals) and lateral sinuses (1 animal)

Venous engorgement and cortical swelling were seen in all animals except those whose superior longitudinal sinuses were occluded anteriorly to the rolandic veins Introduction of radiographic contrast material into the cerebral circulation confirmed the presence of occlusion and demonstrated the development of collateral channels The venous phase of carotid artery injection was employed whenever the superior longitudinal sinus was open but its tributaries were occluded Successful visualization of the collateral venous system by this method occurred in only one instance The animal however expired immediately following the injection of the dye which might account for the radiographic success When muscle was used to occlude the superior longitudinal sinus posteriorly to the rolandic veins (5 animals) (Fig 1) recanalization was demonstrated

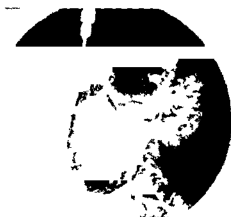


Fig 1 Sinogram of monkey immediately following occlusion with muscle tissue of the superior longitudinal sinus posterior to the rolandic veins Multiple collateral channels can be seen

radiographically in each instance within 2 weeks At this site in other animals after occlusion with ligature or paraffin sinograms repeated in 2 or 3 weeks often demonstrated numerous local collateral routes by passing the obstruction

In 5 animals electroencephalographic studies and determination of changes in the cerebrospinal fluid pressure were carried out in order to obtain further information on the effect of sudden ligation of the superior longitudinal sinus posteriorly to the rolandic veins In each instance

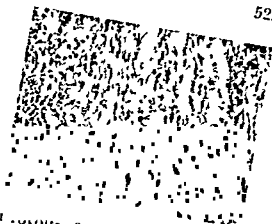


Fig 2 Appearance of muscle occluded dural sinus 2 weeks following intraluminal insertion Note spaces filled with red blood cells (× 120)

high amplitude slow waves (200 to 300 mi...ovolts, 3 to 5/sec.) appeared immediately, followed by a gradual disappearance of the abnormal wave forms and a return to a baseline pattern over a period of time ranging from 20 to 90 seconds Associated with the appearance of the high amplitude slow waves was a rise in cerebrospinal fluid pressure, with increases ranging from 70 to 100 mm H_2O A plateau of 70 to 100 mm H_2O above the pre-occlusion pressure was reached in each instance within 2 minutes These elevated pressure levels had remained unaltered when the experiments were terminated some 4 hours later

There were no deaths related to the occlusion of the dural sinuses and no neurologic deficits were encountered in any of the 28 monkeys

That portion of the dural sinus of 5 animals obstructed by skeletal muscle was excised in all instances at the end of 15 days Microscopic sections of these specimens revealed conversion of the muscle into collagenous scar tissue which appeared to be firmly adherent to the wall of the sinus but which contained endothelial lined spaces filled with blood elements (Fig 2) These appeared to merge into larger channels at both ends of the obstructing mass These findings confirmed the previously demonstrated radiographic recanalization There was no evidence of thrombus formation

Gross and microscopic examinations were made of sinus and brain specimens from animals sacrificed from 5 days to 6 months following the experimental procedures No evidence of neuronal or glial change was found Normal amounts of postoperative scarring were encountered at the sites of sinus occlusion Again no intraluminal thrombus formation was found

DISCUSSION AND CONCLUSIONS

All authors agree that the superior longitudinal sinus can be ligated with impunity anteriorly to the point of entrance of the rolandic veins There is some clinical evidence[2,3] that the dire neurologic sequelae to dural sinus occlusion posterior to the rolandic veins may be temporary, and that the extent and duration of the neurological deficit may depend on the local damage which has been produced The experimental evidence presented here of cortical swelling and venous engorgement, associated with an increase in cerebrospinal fluid pressure but with only transitory changes in the electroencephalographic pattern, indicates temporary alterations similar to those that might be expected in man The apparent rapid development of collateral venous outflow seems to prevent permanent

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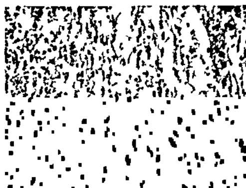


Fig 2 Appearance of muscle occluded dural sinus 2 weeks following intraluminal insertion Note spaces filled with red blood cells (x 120)

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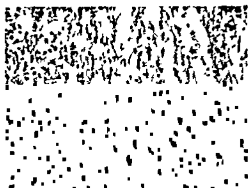


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neurologic alterations in the monkey as well as the dog These experimental findings in the absence of widespread cerebral trauma may lend support to the above mentioned clinical observations

The electroencephalogram in man would appear helpful in assaying the immediate effect of venous occlusion The transient appearance of electro encephalographic abnormalities may well provide a measure of the ability of the collateral circulation to return the pooling venous blood to the heart

The surprising absence of thrombus formation in this study confirms the findings of Beck and Russel[1] who were unable to produce thrombosis of dural sinuses in dogs except in the presence of extensive parenchymal damage from trauma or infection They also observed experimentally that muscle was ineffective in establishing permanent occlusion of the dural sinuses It is again made evident from this study that skeletal muscle is a useful agent in the repair and control of hemorrhage encountered in extensive dural sinus injuries in man

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BLOOD PRESSURE ALTERATIONS DURING TEMPORAL LOBE SEIZURES*

O J ANDY, R McC CHINN AND P BONN

Definite blood pressure changes have recently been demonstrated follow ing stimulation of various structures of the temporal lobe Most of the previous reports have dealt with anesthetized animals and have revealed differences in the character of the blood pressure changes [1 2 3 4] It was thought worthwhile to repeat the experiments in the unanesthetized cat although this study was primarily oriented to determine the effects of temporal lobe seizures on blood pressure controlling mechanisms

METHOD

In a series of 40 cats blood pressure and electroencephalographic record ings were simultaneously made during seizures induced in the amygdala and other surrounding temporal lobe structures Electrical stimulation was carried out with a Grass square wave stimulator having a 1 m sec pulse 30 cycles/sec for a duration of 5 seconds Voltages ranged between 1½

*From the Jackson Department of Surgery Division of Neurosurgery University of Mississippi Medical Center Aided in part by a grant (B 815C) from N I H U S Public Health Service

to 5 volts. Simultaneous blood pressure and electroencephalographic recordings were made on a Sanborn Polyviso recorder and Grass electroencephalograph, respectively. Over 90% of the experiments were done on the awake and unanesthetized animal. Other experiments were done while the animals were under the influence of nembutal anesthesia or succinylcholine and controlled respirations. Following the completion of each experiment the brain was perfused with saline followed by formaldehyde containing potassium ferrocyanide solution. Serial histological sections were made for identification of all points of stimulation and recording.

RESULTS

In general, stimulation of the amygdala and its environs elicited a very definite blood pressure drop. The blood pressure change varied from 5 to 45 mm. Hg. In most instances a fall in blood pressure occurred immediately after the onset of applied stimulation. (Fig. 1). There were 4 different blood pressure patterns during the electrical after discharge: (1) the blood pressure remained below normal throughout the duration of the

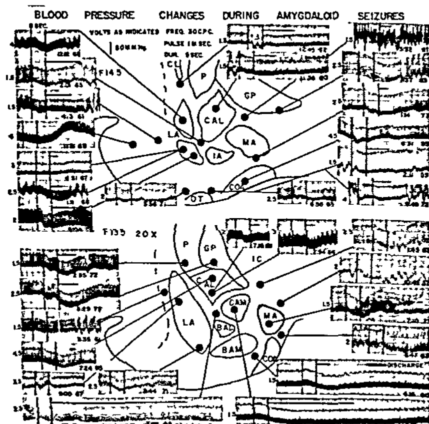


Fig 1 Blood pressure responses are plotted in respect to the specific points of stimulation in 2 different coronal plains of the amygdaloid complex 1 mm apart Voltage is recorded in front of each graph The space between the vertical lines separates the 5 sec interval of stimulation The horizontal bar projecting from the termination of the stimulus represents the duration of the after discharge The numbers in the right lower corner of each run identifies the specific stimulation and experiment The reader must be cautioned against misinterpreting a response such as is demonstrated in #69, II 61, which represents artefact due to movement.

seizure; (2) the blood pressure dropped during the first phase of the seizure and slowly returned to normal before completion of the discharge, (3) very minimal or no blood pressure change occurred during the discharge; (4) definite elevations above the pre-seizure level occurred during the latter stages of the discharge. Terminations of the discharges were usually accompanied by a gradual return to pre seizure levels, although there were incidences of a very definite decline immediately after the cessation of the seizure.

An attempt was made to correlate the various blood pressure responses with the specific temporal lobe structures stimulated. After plotting the results in 12 frontal planes as illustrated by 2 of them in Figure 1, no specific correlation could be drawn between the response and the specific nuclear and fiber masses being stimulated. However, stimulations of the *hippocampus (Fig. 2) had a tendency to either produce no change in blood* pressure or a slight elevation. Stimulations of this structure did not produce blood pressure depressions as did the amygdaloid and pyriform cortex stimulations.

The amygdaloid discharges, in general, were characterized by 2½ to 10/sec. high voltage sharp waves, with a predominance of 4 to 6/sec

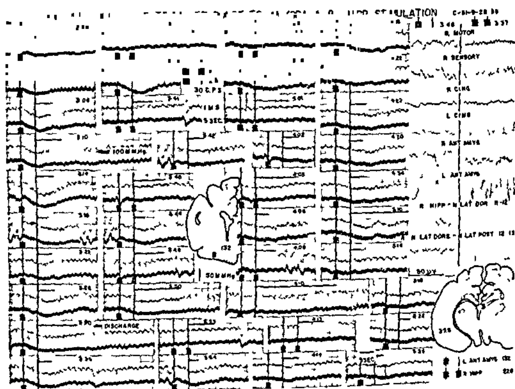


Fig 2 Typical blood pressure tracings during stimulation and after discharge of the left amygdala and right hippocampus are demonstrated Note the marked drop in blood pressure with amygdaloid seizures as compared to the ... There are occasionally ... npare the marked ... with the minimal ... EEG patterns are demonstrated

activity. Discharges were relatively short in duration lasting from 2 to 10 or 15 sec. Occasionally they were much longer. It is of interest to note that the electrical discharges of shorter duration were more inclined to give a greater and longer lasting blood pressure depression, whereas the discharges of longer duration were not accompanied by marked blood pressure drop. The latter primarily consisted of slight fluctuations of blood pressure with minimal depressions and elevations (Fig. 1, seizure 7 52 animal 62). The blood pressure depressions were characterized by a more or less equivalent drop in the systolic and diastolic components. No alteration of the pulse rate was noted during the discharge. Occasionally there was a slight increase of pulse pressure in the seizures of longer duration. Most of the amygdaloid afterdischarges were accompanied by ipsilateral facial movements and occasionally mastication. None of the discharges resulted in major motor seizures.

Hippocampal afterdischarge patterns were characterized by high voltage fast activity as previously described.[5]

DISCUSSION

The present study appears to contradict some previous reports which revealed blood pressure elevations following amygdaloid stimulation. Four possible explanations for the discrepancies might be suggested. (1) Induction of seizures produces obvious disturbances which would not be elicited with stimulations of sub seizure producing thresholds. (2) Stimulation of the amygdaloid and hippocampal structures were either not adequately controlled histologically or the stimulus strength was strong enough to implicate the hippocampus or other related structural systems. (3) The presence of or absence of a general anesthetic might have accounted for the differences. Preliminary studies to eliminate this possibility revealed no essential difference in the blood pressure responses before and after barbiturate induced anesthesia. In addition several animals were immobilized with succinylcholine chloride and artificially ventilated. This was *done to eliminate any possibility of alterations to blood pressure which* may be consequent to changes in respiration occurring in the unanesthetized animals during the amygdaloid afterdischarges. The results from these experiments however were the same as those without curarization. (4) Another possibility for a difference in results may be due to the character of the applied stimulus. Stimuli characterized by low frequency were exclusively employed throughout this study. It is highly probable that higher frequencies as utilized by others may account for the elevation of blood pressure observed.

It is of interest to speculate that a depression of blood pressure which is observed during an amygdaloid afterdischarge may be buffered or restored to normal by propagation of the same discharge to the hippocampal system which has a tendency to maintain normal blood pressure or produce slight elevation. Thus one may look upon these two structures as representing two opposing systems within the temporal lobe potentially capable of influencing blood pressure controlling mechanisms. The most important question is unanswered. That is: What is the mechanism through which a blood pressure depression is induced during an amygdaloid stimulation and afterdischarge? This aspect of the problem is currently under study.

CONCLUSIONS

1 Electrical stimulation and after discharges of the amygdala and its immediately surrounding structures appear to induce a depression of systemic blood pressure in the cat

2 Hippocampal induced after discharges appear to maintain the pre stimulation blood pressure or may produce a slight elevation

3 The longer amygdaloid after discharges have a lesser tendency to produce blood pressure depression than the shorter ones

4 The blood pressure responses following amygdaloid stimulation could not be specifically related to any particular nuclear or fiber mass being stimulated

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AXONAL REGENERATION IN THE TRANSECTED ADULT FELINE SPINAL CORD*

JAMES B CAMPBELL C ANDREW L BASSETT JACOB HUSBY AND CHARLES R NOBACK

A method for obtaining orderly regeneration of axons across a gap in the adult feline spinal cord following complete transection has been the subject of a preliminary report[1] from these laboratories Further experience warrants a more detailed description of the technique and histologic findings At the outset it should be emphasized that clinical trial is not indicated at the present time since no significant alteration in the paraplegic state of the animals has been noted

*From the Departments of Neurological Surgery Orthopedic Surgery and Anatomy Columbia University College of Physicians and Surgeons and the New York Orthopaedic Hospital Columbia Presbyterian Medical Center New York City Supported by grants from the Department of the Army Office of the Surgeon General (Contract No DA 49 007 MD 545) *Playtex Park Research Institute and United Cerebral Palsy Associations Inc* and National Institutes of Health Public Health Service Grant No B 749

With the technical assistance of Mr Lloyd Wenger and Mrs Mary O'Neill

Impetus was given to this research by Windle's[2] recent demonstration that axons can on occasion regenerate in the completely transected spinal cords of Piromen treated animals The work herein reported was undertaken as soon as a method for bridging gaps in feline peripheral nerves became standardized[3] A woven nylon tube impregnated with porous cellulose acetate (H A Millipore) is used to encase the severed proximal and distal stumps of a portion of the sciatic nerve It was believed that a modification of this technique might hold promise for experimental repair of the transected spinal cord since the wall of the tube prevents ingress of connective tissue and provides a scaffold for growth of cells and axons The 0 15 micron openings which constitute 80% of the volume of Millipore permit diffusion of fluids from the tissue bed for nourishment of regenerating tissues The plastic evokes a minimal foreign body reaction and becomes surrounded by a pseudosynovium

METHOD

The surgical procedure is carried out under intravenous nembutal anesthesia and aseptic conditions The first 3 segments of the thoracic spinal cord are exposed by laminectomy and a longitudinal dural incision The dentate ligaments in the middle segment are cut and a curved dental spatula passed beneath the cord When transection is completed the severed ends of the cord retract leaving a 4 mm gap A strip of nylon reinforced Millipore approximately 1 cm wide is passed beneath the proximal and distal stumps No attempt is made to approximate the severed ends of the spinal cord However in certain experiments single or multiple 5 0 Silicone Coated † undyed silk sling stitches were used (Fig 1) In other experiments no sling stitches were used The tube is fashioned by bringing the ends of the Millipore strip together and rolling them to form a cuff secured by silver clips (Fig 2) A single dural stitch passed through the upper and lower limits of the tube provides stabilization No attempt is made *to close the dura One half of the animals received cortisone acetate* 5 mg/kg of body weight daily for 14 days after operation Bladder management consists of bi daily manual expression which is facilitated by bilateral pudendal neurectomy The spinal cord of the control animal was transected and no Millipore sling stitches or cortisone acetate were used

Longitudinal histologic sections were made of the operative sites after survival times ranging from 4 days to 4½ months The specimens were initially fixed *in situ* by intracardiac infusion with 10% formalin and stained with hemotoxylin eosin and by the Romanes and the Bodian silver impregnation techniques

RESULTS

The gaps in the spinal cords of 10 animals which were permitted to survive for 30 days or more after transection were bridged by solid tissue (Fig 3) The plane of transection was not evident by gross examination with a dissecting microscope The width of the bridge within the sheath

†American Cyanamid Company Surgical Products Division Danbury Connecticut and Silastic 9711 Dow Corning Corporation Midland Michigan

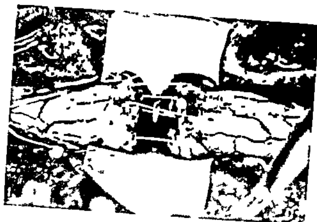


Fig 1 Transection at second thoracic level Multiple sling stitches of Silicone coated silk span the gap Millipore sheet in subdural position

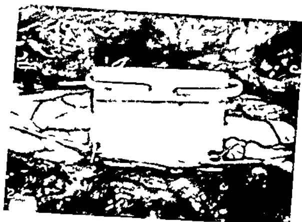


Fig 2 Same as Fig 1 Millipore fashioned into a tube

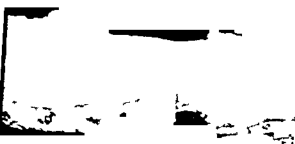


Fig 3 Formalin fixed cord 30 days after transection ventral surface Millipore sheath removed

anged from 1/6 to 2/3 of the adjacent spinal cord This was in contrast o the control animal in which the plane of transection was clearly visible t the end of 30 days

Microscopic examination of the tissues which had regenerated for 30 ays following transection showed groups of linearly arranged axons Imme ately deep to the pia arachnoid complex, the fibers were closely packed, hereas, more centrally, the bridges were composed of loose cellular tissue ntaining fewer axons In the bridges that had regenerated for 10 to 11 eks, the fiber population was markedly increased and uniform through t the sections (Figs 4, 5) In the control animal, an occasional fiber th random orientation could be detected in the mass of cellular reaction

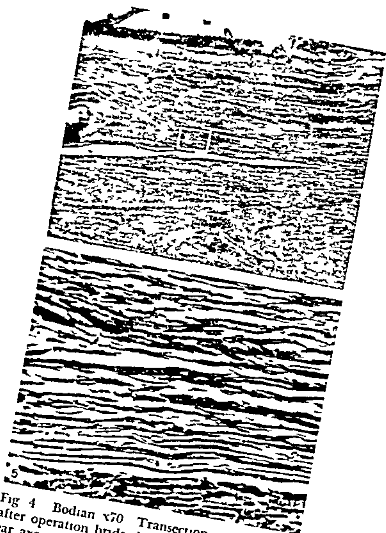


Fig 4 Bodian x70 Transection site 11 weeks after operation bridged with closely packed linear axons Millipore tube wall visible at upper margin Note paucity of cells in bridge except for pia arachnoid complex

Fig 5 Bodian x100 Same as Fig 4 detail from marked area

at the site of transection Constrictive dural scarring and overproliferation of the pia arachnoid membrane was not found in the Millipore protected animals, but was evident in the control Microscopic examination of the spinal cords of two animals dying 4 days after transection indicated that degeneration within the cord extended for 4 to 5 mm in either direction

No return of function has been observed in this study

DISCUSSION

The most striking feature of this research has been the consistent bridging of the transection gap by linearly arranged axons The pattern of regeneration has been essentially the same as that seen in the peripheral nerve study[3] There has been no overproliferation of meningeal or glial elements These findings are in contrast to the control animal and the work of others[2]

It is possible that, in addition to restricting the growth of connective tissue elements from invading the transection gap, the Millipore tube

provides a peripheral scaffold for the pia arachnoid complex Under these circumstances, the transected cord would not be capped by differentiated tissue before axonal regeneration could begin

The use of sling stitches in the transected cord has not been as essential as it is in peripheral nerve regeneration The hurdle imposed by the initial gap in the cord is certainly not as great as that imposed by the 1 to 2 5 cm gaps produced in peripheral nerves However, there was evidence that, because of degeneration of proximal and distal cord segments inside the tube, the gap ultimately spanned by fibers was not 4 mm, but approached 1 cm It is probable that both ascending and descending fibers are participating, because the configurations of the proximal and distal regenerating cord are similar

The intricate morphology and physiology of the spinal cord caution investigators not to anticipate the rate of return of function observed in peripheral nerves Work continues to determine if this technique can be developed to alter the paraplegic state

CONCLUSIONS

1 A readily reproducible technique has been evolved for obtaining orderly, linear, axonal regeneration across transection gaps in the adult feline spinal cord

2 The results of this study largely parallel the pattern of regeneration obtained in previous research with peripheral nerves

3 No return of function has been observed

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THE OPERATIVE METHOD AND PHYSIOLOGIC CONSEQUENCES OF TOTAL HEMISPHERECTOMY IN THE MONKEY*

ROBERT J WHITE, LEON H SCHREINER, ROBERT A HUGHES, COLLIN S MACCARTY, AND JOHN H GRINDLAY

The treatment of overwhelming malignant lesions of the brain remains an unsolved problem[1] Radical operation in the form of hemispherectomy has been attempted in cases of unilateral infiltrating gliomas and recurrent tumors of the brain[1,2] but neoplasms involving the thalamus or sub

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thalamic structures have been considered inoperable because of the possibility that their removal would result in impairment of consciousness or compromise the survival of the patient[2]

Little experimental work has been carried out in the primate to define the anatomic limits of resectability compatible with consciousness or *survival nor have the physiologic consequences of total ablation of a* cerebral hemisphere (including the thalamus) been examined extensively Of importance is Sherrington s work[3] in 1898 and Mettler s[4] in 1943 The latter succeeded in producing a total hemispherectomized monkey however, two separate operations were required for its production and its functional recovery was studied for only 3 months Speaking of the brain of the monkey Mettler[4] has said As a general rule it has not been found possible to remove more than the cortex putamen and caudate nucleus from one side of the brain at one time [2]

Since modern neurosurgical technique had not been utilized in previous studies an investigation was undertaken to study the methods and physiologic effects of removing an entire cerebral hemisphere in the primate

METHOD

Twelve healthy young adult rhesus monkeys with body weights ranging from 3 to 9 kg were subjected to total hemispherectomy which consisted of complete unilateral removal of all cerebral cortex basal ganglia internal capsule and *thalamus at one operation Careful clinical and neurologic* observations were made of these animals before and after the operation

Complete necropsy was performed on each animal that died or was killed The extent of the surgical lesion was verified grossly and microscopically Cinematographic studies were made in order to analyze functional improvement after operation

Operative Technique *Total Hemispherectomy (Fig 1)* Prior to the operation catheters were inserted into the femoral artery for blood pressure recordings and into the inferior vena cava (via a vein in a posterior leg) through which anesthetic agents and fluids could be administered during operation and blood samples could be obtained before and after operation The animal s cardiac response to the surgical procedure was monitored

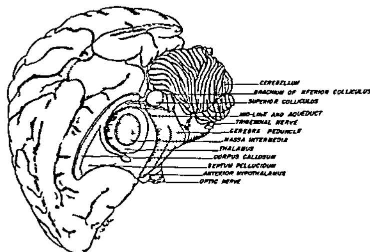


Fig 1 Schematic drawing of a monkey s brain after total left hemispherectomy Shaded areas represent limits of resection

by means of continuous electrocardiographic tracing The amount of blood lost was determined from preoperative and postoperative hemato crit readings

Following the induction of satisfactory intravenous anesthesia with pentobarbital sodium 25 to 35 mg /kg of body weight an aseptic fronto temporoparietal craniotomy was performed and the dura was opened The exposed cerebral cortex was incised with a thin bladed scalpel to a depth of 2 cm The incision was made in the form of a cross extending to the limits of the exposed cortex The occipital lobe was elevated dorsolaterally and removed, and in rapid succession the other portions of the cerebral cortex were removed with a brain spatula and suction When the cerebral cortex had been removed the major cerebral arteries were easily seen and bleeding was controlled with cautery or hemostatic clips The basal ganglia and the thalamus were carefully removed by means of low pressure suction and sharp dissection The third ventricle was opened and tissue lateral to it was removed The distal limit of the excision extended to within 2 to 3 mm of the superior colliculus The ventral limit was through the subthalamus and an attempt was made to spare the hypothalamus When all bleeding was controlled the cavity was closed the bone was wired in place and the wound was closed in layers

Careful postoperative care was directed toward maintenance of body temperature and fluid balance as well as the prevention of infection

RESULTS

All 12 monkeys undergoing total hemispherectomy survived the initial operative procedure however 2 of the animals eventually succumbed to overwhelming meningitis

Physiologic measurements during the actual operation revealed a gradual fall in blood pressure to hypotensive levels (approximately 1/3 to 1/6 of the preoperative value) At the time of skin closure the blood pressure had risen to approximately 2/3 of the preoperative level The electrocardiogram displayed only minimal changes even during hypothalamic manipulation The maximal changes occurred during cortical incision and were mani fested by atrioventricular conduction block The venous hematocrit read ing fell rapidly during the immediate postoperative period within 20 hours of surgery the hematocrit readings averaging 8 to 10 percentage points lower than preoperative values were recorded

Immediately after recovery from anesthesia (usually within 2 to 4 hours after operation) the monkeys demonstrated (1) wakefulness and awareness of their environment and (2) marked flaccidity paralysis insensitivity to painful stimulation and depression of muscle stretch reflexes of the con tralateral or involved extremities i e the extremities contralateral to the side of the operation

In spite of the overwhelming unilateral loss of brain tissue each animal rapidly regained consciousness and normal behavior patterns of eating and grooming Within 2 weeks of operation the animals required no more care than normal monkeys As motor function improved they became investigative and aggressive but never completely regained the furtive behavior of an untamed rhesus monkey

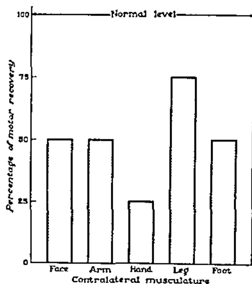


Fig 2 Recovery of motor function in the contralateral musculature in the total hemispherectomized monkey

Although no isolated movement or reception of painful stimulation was elicited in the contralateral limbs during the first 24 hours after operation, muscle function and sensory reception gradually returned in these extremities (Fig 2) Within 2 to 3 months, excellent recovery of motor function had been attained, the animal was able to stand without support, and run and climb with amazing agility While these grosser muscular activities were performed well contralaterally, tasks requiring fine manipulations of distal musculature were never accomplished This is reflected in the poor (25%) motor recovery seen in the hand as opposed to good (75%) functional recovery in the leg

By one month after operation the monkeys were able to appreciate well painful stimulation in the form of pinching or heat or both contralaterally Whereas the reception of painful stimuli was considered 75% of normal with good localization over the contralateral portion of the face, sensory appreciation was considered poor (25%) in the contralateral arm, leg and portion of the trunk Heat appeared to furnish a more painful experience than pinching Light touch vibratory sensation, position sense, and placing reactions were measurably absent contralaterally to the site of operation on the involved side Motor and sensory function was considered normal on the same side of the body as the resection

The early flaccidity of the contralateral extremities gradually lessened, particularly with regard to the upper limb Here flaccidity changed to spasticity of limited degree, most noticeable at the elbow, where a semiflexor position was assumed and minimal to moderate passive resistance was observed The contralateral leg continued to demonstrate minimal flaccidity

Following the initial depression of the contralateral muscle stretch reflexes minimal hyper reflexia gradually developed This was easily demonstrated in the contralateral patellar reflex, which, while remaining hyper reactive gradually approached but never equaled its preoperative state

A Babinski response has been reported in the monkey following section[5] of the cord and also ligation of the anterior cerebral arteries[6] None of our monkeys had a Babinski response On plantar stimulation no move

ment of the toes or foot was the usual contralateral finding, while a marked withdrawal of the foot and toes was observed ipsilaterally

Examination of animals 1 to 2 years after total hemispherectomy re affirmed the preservation of their psychologic and neurologic attainments

DISCUSSION

This technique of cerebral extirpation represents a marked departure from the methods of large cortical and subcortical ablation in the primate reported in the literature[7] The method of extirpation used in these animals more closely approximates the operative procedures of Bazett and Penfield[8] in the production of unilateral decerebrate cats However, in our animals no attempt was made to reduce cerebral blood flow during total hemispherectomy, sole reliance being placed on the normal physiologic mechanisms of controlling loss of blood following arterial division This is immediately observed as marked vasoconstriction of the major cerebral arteries, and has been sufficiently successful in reducing loss of blood during cerebral ablation so that surgical death has not been seen in these animals

In spite of the overwhelming unilateral loss of cerebral tissue (Fig 3) it appears that total hemispherectomy in the monkey does not interfere with the level of consciousness This is an interesting finding when con sideration is given to the disturbances of consciousness and the production of coma associated with tumors or infarctions of portions in the human brain[9] *similar to those removed from these animals*

The recent work of French and Magoun[10] on the activating or ascending reticular system of the brain stem (nuclear patterns of which extend as far forward as the thalamus, subthalamus and hypothalamus) has demon strated that lesions in this system result in hypersomnolence and extensive destruction of this region is not compatible with life in the monkey Exam ination of our surgical specimens revealed that dorsal portions of the hypothalamus may not escape resection It seems therefore, that as long as a lesion is completely unilateral in its involvement of the anterior reticular system the elements of consciousness remain intact



Fig 3 Microscopic section of an operative animal s brain at the level of the thalamus lateral geniculate body and optic chiasm Note open third ventricle reduction in left hypothalamus and left thalamus (Weil s stain x6)

The excellent restoration of motor function following total hemispherectomy appears to give credence to the theory of bilaterality of motor innervation While it is true that somatic responses have been elicited from the subthalamic and hypothalamic regions of the monkey by Ectors [11] and theoretically could give rise to the purposeful movements on the contralateral side from the hemispherectomized preparations the high degree of functional attainment seems to implicate cortical influence Bilateral movements following cortical stimulation have been elicited in the monkey by Bucy and Fulton [12] and Wyss [13] Fulton and Sheehan[14] have demonstrated histologically uncrossed lateral pyramidal tracts in the monkey

Fulton [15] in discussing the residual sensibility in hemispherectomized human beings and hemidecorticate monkeys stated that the remaining crude sensation is due to both ipsilateral thalamic representation and contralateral residual thalamic function In our totally hemispherectomized *monkey the unilateral thalamus has been extirpated also* Our studies seem to answer the question in favor of ipsilateral sensory representation in the thalamus

CONCLUSION

A total cerebral hemisphere including the thalamus can be removed successfully from the brain of the rhesus monkey at a single operation without interference with consciousness and with only moderate reduction in motor and sensory function

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ANGIOGRAPHIC STUDY OF INTERNAL CAROTID BIFURCATION*

O J ANDY AND JAMES S BROWNE

Angiographically the anteroposterior view of the internal carotid bifurcation often presents marked variations from a preconceived normal In order to evaluate more adequately these deviations, anteroposterior arteriograms were performed during contralateral carotid artery compression By this technique it was possible to compare the patterns of the right and left internal carotid bifurcations

A series of 31 cases were collected for this preliminary report Patients ages ranged from 14 to 68 years Technique consisted of injecting 10 cc of 50% hypaque in one common carotid artery while the opposite artery was manually compressed The x ray tube plate distance is 40 inches and angled at 20° to 25° toward the foot of the table

As seen in Figure 1, there is a marked variation in the patterns of the internal carotid bifurcation In addition to a wide variation between

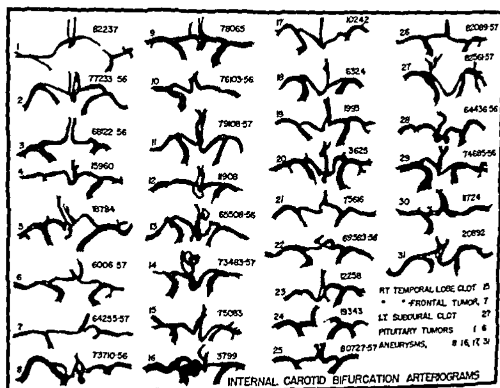


Fig 1 Cerebral arteriogram tracings of the anteroposterior view of the internal carotid bifurcation X ray tube plate distance was 40 inches and angled at 20° to 25° toward the foot of the table These are arranged according to decreasing intercarotid bifurcation distances Note the marked variation in pattern and size of the vessels The variability in so termed normals becomes more impressive if one attempts to identify the pathological tracings before referring to the inserted descriptive legend

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any two individuals, there often is a definite variation between the 2 patterns within the same patient. An attempt was made to find a significant angle between the 3 major vessels at the bifurcation, but marked variation in patterns made this impractical. Alterations in the vascular configuration had to be extreme in order to indicate significantly the presence of a pathological lesion. This is well illustrated in Fig. 1, No. 1, which was a large pituitary tumor extending well beyond the limits of the sella turcica. Fig. 1, numbers 7 and 27 also represent large lesions. In No 27 the shift is great enough to produce a superimposition of horizontal segment of the right anterior cerebral over the internal carotid Variations of a lesser degree as in the normals, numbers 5 and 28 in Figure 1, often present difficulties in interpretation.

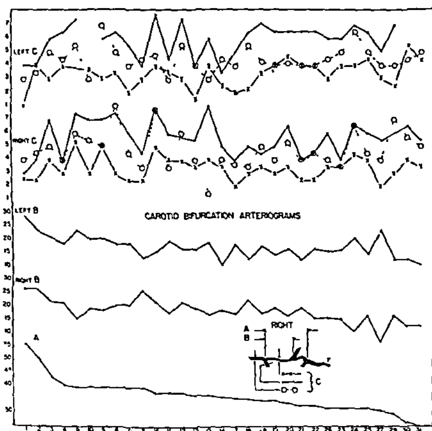


Fig 2 As illustrated in the diagrammatic insert, (*A*) represents measurements of the intercarotid bifurcation distance in order of decreasing measurements, (*B*) represents the distances between the internal carotid bifurcation to the junction of the ascending portion of the anterior cerebral arteries, (*C*) represents diameter measurements of the internal carotid taken within 2 to 3 mm from the bifurcation and the narrowest diameter of the transverse branch of the anterior cerebral arteries and middle cerebral arteries Right and left sides of *B* and *C* are compared This entire graph stresses the marked variability existing between the sizes and patterns of the 3 major vessels making up the internal carotid bifurcation Points in the graph representing pathology can be identified by referring to Fig 1 Measurements of the diameters are in terms of thousandths of a foot and distances in mm Case numbers are represented along the abscissa

Distances between the carotid bifurcation and the ascending segment of the anterior cerebral arteries were compared with the intercarotid bifurcation distance as illustrated in Figure 2, A and B The interbifurcation distance among the normals varied from 22 to 51 mm It is interesting to note that the pituitary tumor (No 1, Figs 1 and 2), with extrasellar extension, produced an interbifurcation distance of 56 mm This was obviously produced by expansion of the sella with resulting lateral displacement of the carotid siphons In most instances the distance from the carotid bifurcation to the anterior ascending cerebrals is approximately 50% of the interbifurcation distance However No 18, Figs 1 and 2 although normal, represents a definite deviation from this observation The greatest variation is found in No 27, Figs 1 and 2 which represents a left subdural hematoma

The diameters of the 3 major vessels were compared and found to present a definite variability The middle cerebral artery presents the greatest variability when compared to the internal carotid and anterior cerebral The anterior cerebral artery is invariably smaller in width than the internal The middle cerebral artery may vary between larger than the internal carotid or smaller than the anterior cerebral arteries In the normal arteriograms the homologous vessels of opposite sides reveal as much as 50% variability in size This could easily be confused with spasm or arteriosclerotic narrowing (No 5, Fig 1)

CONCLUSION

The anteroposterior views of the internal carotid bifurcation were studied in 22 'normal and 9 pathological arteriograms In general a great variability in the pattern, size, and shape of the vessels was described and illustrated In accordance with the observations, one is inclined to believe that it is extremely hazardous to interpret moderate degrees of narrowing and vessel displacement as pathologic in the anteroposterior views of the internal carotid bifurcation

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USE OF INTRACAROTID ARTERIAL PROCAINE DURING CRANIAL ARTERIOGRAPHY*

WILLIAM F BREHM ARTHUR B KING, JOHN B COUGHLIN, AND JACK A YOUNG

The injection of most radiopaque contrast media into blood vessels causes immediate vascular spasm probably by local irritation, which is often followed at varying intervals by peripheral dilatation[1] This vascular

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Dr J T Littleton assisted us in reading and interpreting all films involved in this study

constriction can make radiography of the blood vessels unsatisfactory, particularly in the case of the cerebral vessels The vessels in the second radiograph in a stereo pair are often poorly visualized due to the spasm following the first injection of contrast media In addition there is involved an unknown degree of hazard to the brain by the resultant spasm of cerebral blood vessels

Over a period of 5 years we have tried to prevent cerebral vascular spasm during angiography by premedication with papaverine The effect and value of this program was not easily evaluated and it may have been a useless therapeutic gesture To evaluate the role of stellate block for the release of cerebrovascular spasm during arteriography stellate block was performed following the first injection of contrast media in 10 cases where stereoradiographs were made No obvious cerebral vessel dilatation or relaxation of spasm was found in the second radiograph of the stereo-pair The vessels appeared the same in the second radiograph or were smaller than in the first radiograph of the stereo pair

The nonrelease of cerebral artery spasm following stellate block led us to abandon this program It also contradicts the contentions of some physicians who advocate stellate block for the supposed vascular spasm associated with cerebral embolism thrombosis or vessel rupture The anatomical demonstration of cervicothoracic sympathetic nerve supply to cerebral vessels is controversial so it seems unlikely that block of the stellate could influence vessel size Hakim and Fisher[2] were not able to observe cerebral vessel constriction when the cervical sympathetics were stimulated while observing cerebral vessels directly under a microscope during craniotomy

It was postulated that the principle of release of spasm of vessels by intravascular procaine might be applied to prevent the spasm of cerebral vessels due to the contrast media To ascertain if procaine could be injected into the carotid arteries in therapeutic amounts without convulsions or other deleterious effects a series of intracarotid injections of procaine were done on 30 rabbits The data from these rabbit experiments revealed that 15 mg/kg of procaine could be injected into rabbit

Table 1 Age Incidence of Arteriograms

AGE	NO OF PATIENTS
0 10	6
11 20	8
21 30	12
31 40	14
41 50	27
51 60	17
61 70	11
Total	95 patients†

†5 patients were done twice making a total of 100 Youngest 2 yrs oldest 68 yrs

internal carotids, if the animals were protected by previous intravenous pentobarbital in sleeping doses With this basic data as weak assurance procaine in small doses and later in larger doses was injected into the internal carotids of 100 humans during the performance of transcutaneous carotid cerebral angiography under thiopental, nitrous oxide oxygen anesthesia Table 1 shows the age distribution of the patients

METHOD

After anesthesia was established, the site of the skin puncture over the common carotid artery was infiltrated with local anesthesia An 18 gauge needle attached to a stopcock and syringe of 2 5% sodium citrate solution was introduced into the common or internal carotid artery Upon successful puncture, the citrate was injected to clear the needle and another syringe containing 25% hypaque was substituted and the first of a stereo-pair of lateral radiographs of the head was made More sodium citrate followed the contrast media A syringe of 1% procaine was substituted and it was injected while observations of the pulse, blood pressure, respirations and pupil size were made About 1 to 2 minutes following completion of the procaine injection, 10 cc of hypaque were injected and the second radiograph of the stereo pair was made The films were later viewed to ascertain if the vessels following procaine were larger and incidentally for the presence of any intracranial lesion The first case was given a 50 mg dose, the second 75 mg, the next 5 cases, 100 mg, and all the rest, 200 mg at each injection If the patient was a child, a smaller dose was given It was found that children could tolerate a procaine dose out of proportion to their weight and size because of the rapidity of cerebral circulation and the fact that a child s head is larger in proportion to his body than is an adult's If bilateral arteriography was performed, the same dose was given on both sides

On injecting the third case, it was discovered that if the needle was in the correct position in the internal carotid, the ipsilateral pupil dilated within 30 seconds after starting the procaine injection, which usually took about 15 to 20 seconds Often the pupils began to dilate during the injection of 20 cc. of 1% procaine Pupil dilatation persisted as long as 20 minutes but was sometimes less It appeared that pupil dilatation was a test for correct placement of the needle, and this will be discussed later

RESULTS AND DISCUSSION

We were apprehensive lest, by the injection of procaine directly into the circulation of the brain, we would induce circulatory collapse and convulsion – the classic signs of the so called procaine reaction It was soon obvious that the dose of 200 mg of procaine directly into the brain did not produce circulatory collapse

In 73 of the 100 cases, no significant change in blood pressure or pulse occurred In 21 cases the blood pressure rose, accompanied by an increase in pulse rate The greatest rise was 60 mm Hg while the usual rise was 10 to 20 mm Hg Blood pressure decreased in 6 cases, the greatest decrease being 40 mm Hg, but the usual drop was 10 to 20 mm Hg In 2 cases blood pressure rose 20 mm Hg when procaine was injected into the

opposite carotid after a slight decline in blood pressure had occurred when the first side of a bilateral arteriogram had been done.

In only 1 case of the 100 was it deemed advisable to use a vasopressor to correct hypotension; 5 mg. of d-desoxyephedrine was given intravenously to a patient whose original pressure of 100/80 declined during the procedure to 80/60 following injection of the first side of a bilateral arteriogram. Injection of the contrast media and procaine into the second side was without incident.

As with blood pressure, the pulse rate in most cases did not change significantly. In 16 cases the pulse rate increased and in 2 cases the pulse rate decreased. The usual increase was 10 to 20 beats per minute, but one case had an increase of 50 beats which was accompanied by a rise of 50 mm. Hg in blood pressure. It was postulated that the increase in pulse and blood pressure was probably a result of release of the carotid sinus mechanism by internal anesthetization.

In most cases changes in respiration were not obvious to casual observation. However, 32 cases showed observable hypopnea or actual apnea. The change in respiration occurred at about 1 to 1½ minutes after injection of procaine was started. The longest apnea was 9 minutes, but the usual interval was 1 to 3 minutes. There were 9 cases of hypopnea and 22 cases of apnea. During the period of depression, respirations were assisted by manual compression of the breathing bag.

As mentioned above, dilatation of the ipsilateral pupil following intracarotid procaine appeared to be an infallible test for correct placement of the needle in the internal carotid artery which would result in satisfactory arteriograms of the cerebral circulation. Twenty-nine cases did not show pupil dilatation following procaine injection. The x-rays proved 26 of these were due to malplacement of the needle and the procaine and contrast media were injected into the external carotid artery or extravasated into the neck. Two cases showed no dilatation but satisfactory intracerebral angiograms were made. This may have been due to shifting of the needle incident to change of syringes. One case showed no dilatation, the contrast media was in the internal carotid but diffused rapidly through massive arteriovenous fistulae. The change in vascular hydrodynamics probably prevented an effective concentration of procaine from producing the desired effect.

In 2 cases there was pupil dilatation but no contrast media reached the cerebral vessels. One case had a thrombosis of the internal carotid distal to the ophthalmic artery. The other occurred without explanation except the possibility of shifting of the needle within the common carotid while changing syringes. Therefore, only 4 of 100 cases failed to confirm the pupil dilatation phenomena as a positive test for correct placement of the needle in preparation for injection of the contrast media.

In the 29 cases where no pupil dilatation occurred, the procaine test was used to determine needle position in subsequent attempts at carotid artery puncture. This permitted or caused multiple 200 mg. doses of procaine to be administered into the vessels within the carotid sheath. The greatest amount of procaine used in a single patient was 1200 mg. The others received from 400 to 1000 mg. during the course of the pro-

cedure No severe reactions occurred during these cases in spite of the large total amount of procaine used in these repeated injections

Our greatest fear was the possibility of convulsion when procaine was injected directly into the cerebral circulation This occurred first on the 14th case and some evidence of cortical irritation occurred in a total of 10 cases It was manifested variously by simple contralateral spasticity of an arm and/or leg to actual mild convulsive movements of the arm and/or leg The seizures were short in duration and none were judged severe or dangerous to the patient All occurred in patients to whom less than 350 mg of thiopental had been administered Two small adults showed no convulsive trends after as little as 250 mg of thiopental had been given with nitrous oxide and oxygen for anesthesia

The actual effect of procaine on cerebral vessel size was difficult to evaluate Inasmuch as procaine will positively release the spasm of peripheral arteries and veins, when injected into them, one could assume that the same local effect would occur in cerebral vessels In 46% of the cases it was believed that the cerebral vessels were demonstrated better on the radiograph when procaine preceded the hypaque Figure 1 is an example of the better demonstration of vessels The right radiograph was taken after the procaine injection In the remainder of the cases it was not possible to see any difference in blood vessel demonstration after procaine In no case was the after procaine radiograph of poorer quality than the radiograph with just the contrast media

Early in the series, ophthalmoscopic examination was made on 10 cases *following injection of procaine Dilatation of the pupil showed* the retinal vessels to be dilated This is no proof that the cerebral vessels were dilated at the same time

Many factors enter the situation in the production of good cerebral arteriograms Our method of evaluating the value of procaine as a dilator or releaser of spasm as based on apparent vessel size on x ray film is subject to error in judgment of the viewer Other technical factors could cloud the proper evaluation of the before and after radiographs

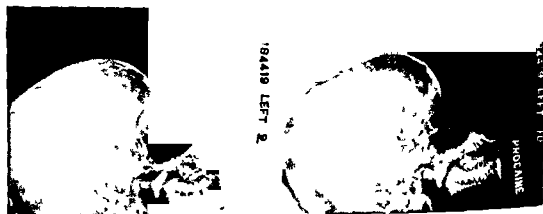


Fig 1 Figure on left shows results of injection before procaine Figure on right demonstrates vessels have larger caliber after 20 cc. of 1% procaine has been injected Both films taken after 10 cc of 25% Hypaque were injected through an 18 gauge needle Time interval between pictures about 2 minutes

We feel this procedure is not accompanied by the adverse effects one might expect in the light of our past fears regarding introduction of procaine into the circulatory system and, particularly, directly into the cerebral circulation Since we have demonstrated that it can be done safely, we enjoin other investigators who are equipped to measure cerebral blood flow and cerebral vascular resistance or to observe directly the blood vessels of the brain to prove or disprove the value of procaine as a cerebral vascular dilator when injected by the intracarotid route

If cerebral vessel spasm accompanies cerebrovascular accidents, and if such spasm is released by procaine, it is interesting to speculate as to the therapeutic possibilities of procaine introduced via the intracarotid route in such cases Four cases of cerebral embolism and/or thrombosis were treated in this manner with what appeared to be better than usual recovery The discussion of these cases is outside the scope of this paper, however, and they were not included in the 100 cases reported

SUMMARY

1 In a test in 30 rabbits, we found that 15 mg /kg procaine could be injected into the cerebral circulation of rabbits if they were protected by previous intravenous pentobarbital

2 Thereafter, 200 mg of procaine at a dose were injected into the carotid cerebral circulation of 100 humans during angiography

3 Mild but insignificant convulsions occurred in 10 cases but never if more than 350 mg of thiopental had been previously administered

4 Circulatory collapse did not occur but often pulse and blood pressure rose probably due to release of the carotid sinus mechanism

5 Hypopnea or apnea of 1 to 9 minutes duration occurred in 32 cases but this was easily overcome by respiratory assistance with the anesthesia machine

6 Ipsilateral pupil dilatation following injection of procaine into the *internal* carotid is a *96% positive test for correct placement of the needle* for cerebral arteriography

7 We believe procaine prevents vessel spasm incident to injection of contrast media and improves the quality of cerebral angiography

8 The use of intracarotid injections of procaine as an adjunct to treatment of cerebrovascular accidents is suggested but not discussed

9 Other investigators with equipment for measurement of cerebral flow are enjoined to test the dilating effect of procaine on cerebral vessels

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EFFECT OF HIPPOCAMPAL SYSTEM AFTER DISCHARGES UPON LEARNED BEHAVIOR*

O J Andy, R McC Chinn, and P Bonn

Ablation studies of the temporal lobe in the monkey have implied that various parts of this structure are concerned with retention and acquisition of discrimination responses Recently in man, the tendency has been to assign some aspects of memory function more specifically to the hippocampus In order to evaluate more readily this particular aspect of hippocampal function, a series of experiments were carried out on conditioned monkeys This is a preliminary report on the results from the first monkey which was studied over a period of 13 months The question to be answered was what effect does a hippocampal system after discharge have on learned visual discrimination?

METHOD

A. Conditioning Technique. Over a period of 7 months using a Wisconsin testing apparatus an adult Rhesus Macaca monkey was conditioned to discriminate between paired colored and form blocks Frequency of testing was 2 to 5 days per week Technique consisted of placing raisins under one of the paired colors in a random series of 10 trials for each of 4 paired colored blocks (gray brown, yellow blue, red green, black white) and paired black white form blocks (square stripe) After the monkey acquired a stable performance level of better than 98% in total of 4650 trials, the first set of subcortical electrodes was inserted

B. Operative Procedure 1. Under local anesthesia and aseptic conditions bipolar concentric stainless steel electrodes were placed in various subcortical structures Interelectrode distance was 1 to 1½ mm They were fixed to the skull with dental acrylic

Operative Procedure 2 Six months after the first operation a set of bipolar cortical electrodes was inserted under similar conditions Daily testing was resumed immediately after each of the operations When the animal returned to the preoperative performance level (4 days after first operation 6 days after the second) combined testing and electrical stimulation and recordings were instituted

C Stimulations and Recordings Electrical stimulation and recordings were done through bipolar needles Stimulus source and character were a Grass square wave stimulator 1 msec pulse 30/sec for a period of 5 sec Voltage varied from 3 to 5 for subcortical and 2 to 30 for cortical structures Electrical recordings were made on an 8 channel Grass electroencephalograph During combined testing and electrical stimulation discrimination blocks were simultaneously presented with the application of the electrical stimulus Electrical recordings were made immediately after completion of each 5 sec period of stimulation Total number of stimulations were 279 (127 cortical 64 of which were seizures and 152 subcortical 44 of

*Department of Surgery Division of Neurosurgery University of Mississippi Medical Center Jackson Aided in part by a grant (B 815 C) from National Institutes of Health U S Public Health Service

which were seizures) Three induced seizures involved loss of consciousness with clonic tonic movements and the remaining 105 were not characterized by such changes

D Histology. At the completion of this study the brain was perfused with solution of saline followed by formaldehyde containing potassium ferrocyanide Serial histological sections were then made and the points of stimulation and recording were identified

RESULTS

Left hippocampus (Fig 1) was stimulated 35 times with 21 resulting in seizures varying in length from 2½ to 30 sec In all but one instance the electrical after discharge appeared to be confined to this structure The discharge pattern consisted of a sustained train of 7 to 25 sec. high voltage sharp waves Predominating frequencies were 9 and 20/sec The electrical activity in the single instance of spread to the opposite hippocampal system was essentially similar Fifty percent of the stimulations was accompanied by closure of the left eye and drawing of the face to the left during the application of the stimulus No facial movements occurred during the application of the after discharge There were no obvious postictal be

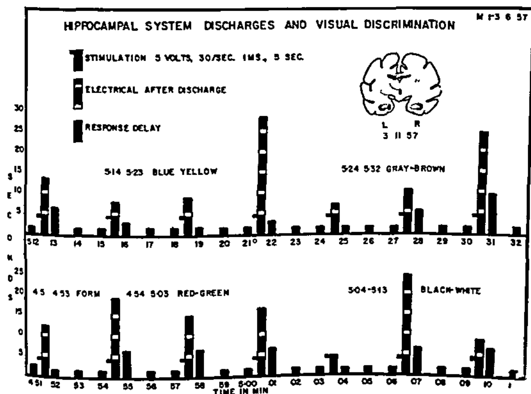


Fig 1 Delay in visual discrimination responses to paired forms and color are plotted in terms of seconds (ordinate) every minute (abscissa) as the monkey was consecutively tested Every third discrimination test was accompanied by a simultaneous electrical stimulation of the left hippocampus (histological insert) The duration of the after-discharges is plotted above the stimulations All choices made during this series were correct Note the minimal effect of the hippocampal system discharge on the response delay compared to that of the frontal lobe discharge in Fig 2

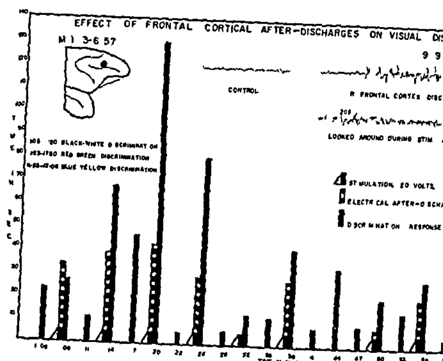


Fig 2 The discrimination response delay is plotted for consecu minute interval trials Right frontal electrical stimulation and after discharges, which were simultaneously induced with alternat ination trials are represented with the associated discrimination delay Note prolonged delay following frontal lobe after disc compared with hippocampal discharges in Fig 1

havioral changes The presence of a discharge and its termi only be detected electroencephalographically

Discrimination tests were performed at 1 minute intervals one of which was accompanied by a simultaneous electrical of the hippocampus (Fig 1) In this series, all but one of the di tests were performed without error In general the animal pe as efficiently and quickly as without the induced seizure It i interest to note that the 50% occurrence of left eye closure a drawing did not prevent the animal from making correct choic it did delay him 2 or 3 seconds The one incident of inco occurred 45 seconds after the completion of an 8 second seizure on the first postoperative stimulating day, and the choice w black and white which made up ⅓ of the 20 preoperative incorr It was felt that this error was one of chance and not directly to the after discharge

Twenty seizures induced on the right side and primarily inv limbic system bilaterally induced no errors in discrimination

The greatest change in the discrimination performance test duced during seizures induced in frontal cortex This primarily of a response delay As demonstrated in Figure 2, there was tendency for response delay to be longer with seizures of longer It must be emphasized that in spite of this delay in response with the electrical discharge the choices were invariably correct these periods of delay, the animal appeared to stare into space, he still exhibited startle reaction and visual perception On 2

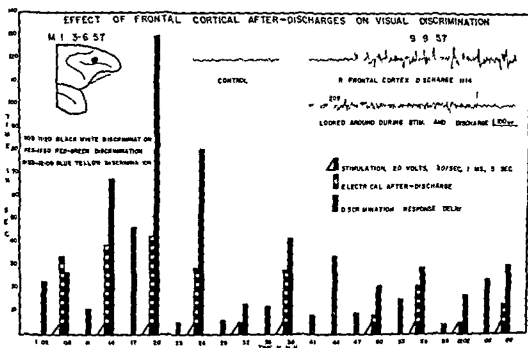


Fig 2 The discrimination response delay is plotted for consecutive three minute interval trials Right frontal electrical stimulation and resulting after discharges which were simultaneously induced with alternate discrim ination trials, are represented with the associated discrimination period of delay Note prolonged delay following frontal lobe after discharges as compared with hippocampal discharges in Fig 1

havioral changes *The presence of a discharge and its termination* could only be detected electroencephalographically.

Discrimination tests were performed at 1 minute intervals every third one of which was accompanied by a simultaneous electrical stimulation of the hippocampus (Fig 1) In this series, all but one of the discrimination tests were performed without error In general the animal performed just as efficiently and quickly as without the induced seizure It is of further interest to note that the 50% occurrence of left eye closure and left face drawing did not prevent the animal from making correct choices, although it did delay him 2 or 3 seconds The one incident of incorrect choice occurred 45 seconds after the completion of an 8 second seizure This was on the first postoperative stimulating day, and the choice was between black and white which made up ⅓ of the 20 preoperative incorrect choices It was felt that this error was one of chance and not directly consequent to the after discharge

Twenty seizures induced on the right side and primarily involving the limbic system bilaterally induced no errors in discrimination

The greatest change in the discrimination performance tests was pro duced during seizures induced in frontal cortex This primarily consisted of a response delay As demonstrated in Figure 2, there was a definite tendency for response delay to be longer with seizures of longer duration It must be emphasized that in spite of this delay in response associated with the electrical discharge the choices were invariably correct During these periods of delay, the animal appeared to stare into space, however, he still exhibited startle reaction and visual perception On 2 occasions

the monkey did not delay beyond the termination of the seizure A major motor seizure lasting 90 seconds was induced in the left frontal lobe It is interesting to note the delay in performance was 6 minutes after completion of the seizure following which all color discrimination tests were performed correctly Discharges induced in the frontal lobe did not spread to other cortical areas (temporal parietal, occipital, and orbital)

DISCUSSION

Although this is the first in a series of animals to be studied and the data is thus inadequate to draw major conclusions, one is still tempted to discuss the results Previous studies done by others[1 2 3 4 5 6 7] indicate that visual discrimination is definitely altered by temporal lobe ablation More specifically[7] ventral hippocampal ablation was found to produce marked impairment of visual discrimination In view of those publications one is hesitant to accept the results of this study However, the present monkey demonstrated no difficulty in the discrimination testing during the induced hippocampal seizures If these results are acceptable, one is thus forced to conclude that either the after discharges involving the hippocampal system do not functionally ablate that structural system or the functional ablation of that system (if conceded to exist) is not equivalent to the results reported from anatomical ablations

Delay in the discrimination responses during frontal lobe discharges are in accord with the results of aluminum hydroxide cream application[8] As previously mentioned by others the alteration in response appears to be due to factors other than disruption of visual function and motor performance

CONCLUSIONS

1 The results of this study suggest that hippocampal system after discharges do not alter learned color and form discrimination responses

2 Frontal lobe discharges produce a temporary delay in the response but do not alter the accuracy of the discrimination

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THE LOCAL EFFECT UPON BONE, CARTILAGE AND JOINT TISSUES OF THE POLYMERIZATION OF METHYL METHACRYLATE IN SITU*

HARRY T GLASER

Various preformed acrylic prostheses have been introduced into and tolerated by living tissues Bone has been found to develop around such implanted materials The sterilization of methyl methacrylate by ultra violet light has made it feasible to mix and mold the material in the plastic form into bone defect, as in the skull The replacement of removed intervertebral discs by locally polymerized acrylic has been proposed by Cleveland [1]

It was considered desirable to study the effect upon tissues in which the polymerization occurs The material available for such purposes consists of liquid and powdered forms † The powder is made up of several methyl methacrylate polymers and co polymers, with other resin forming com pounds with a catalyst, benzoyl peroxide, to promote polymerization The *powder contains micro organisms that in the properly prepared samples* are killed by ultraviolet irradiation The liquid consists chiefly of methyl methacrylate monomer and contains hydroquinone which prevents pre mature polymerization of the liquid It also contains a tertiary amine which promotes self curing of the mass formed by mixing the liquid and powdered methacrylates According to Woringer[2] the liquid material has a pH value so low that it is capable of cauterizing living tissue it might contact

When the liquid and powder are mixed they form a putty like mass, the plasticity of which depends upon the proportion of the two forms During this stage the material may be molded into the shape desired, or if made sufficiently fluid, may be injected into cavities

While in the plastic state the material polymerizes, at which time molecules of methyl methacrylate combine with themselves to form long chains which in turn cause the liquid monomer to produce a solid, hard, polymeric form of methyl methacrylate, liberating a great deal of heat, the temperature reaching 80°C (176°F) At this stage the material is "*cured" and chemically resembles the powder originally employed* The time required for polymerization varies between 8 and 15 minutes de pending upon the proportions of the two fractions mixed

The physical effects of the polymerization, acidity and heat, upon the tissues have been circumvented in surface applications of the material by isolating the mass in plastic bags and by artificial cooling It seemed

†Cranio plastic powder and liquid The D L Caulk Co, Milford Del

*From the Buffalo General Hospital and the Departments of Neurosurgery and Pathology University of Buffalo Medical School Buffalo New York Aided by the Neurosurgical Research Fund of the Buffalo General Hospital

With the technical assistance of Miss Helen Sanderson and Mr Lee Bowerman

desirable to study the effect of the reaction upon tissues, particularly cartilage and bone, within which polymerization might occur directly. Specifically we were interested in the effects of such reactions within the intervertebral disc spaces.

Since the intervertebral disc spaces of experimental animals are too small for easy manipulation, the reaction was studied in the knee joints of the hind legs of a series of dogs. The joint space was opened aseptically and 3 to 3.5 cc. of the acrylic mixture was introduced and allowed to "cure" *in situ*. The wound was then closed surgically. As a control, the contralateral joint was opened similarly, the joint surfaces moderately scarified mechanically and the joint closed. To see the effect of the reaction in bone alone, specimens of the acrylic mass were introduced into defects made into the spine of the scapula of one animal and into the femur of another. Upon healing of the wounds, the motion of the joints containing acrylic was relatively limited, but in all cases after the discomfort due to the local surgical wound had subsided, weight was borne on the limb with only a minimal limp.

The animals were sacrificed at intervals from 1 week to 8 months. The "treated" joints and their controls were excised and fixed in formalin. They were split with a saw and photographed. They were then decalcified, the acrylic mass removed and histologic slides were prepared. The third month specimen was discarded because of infection and that of the sixth month was lost.

HISTOLOGIC EXAMINATIONS

1754. First week. (Fig. 1). There are marked changes of synovitis, with fibrinous-cellular exudate especially in the posterior part of the capsule, which is filled out by this exudate. It is, in part, firmly adherent to the surface epithelium and in a state of beginning organization. Free in the lumen there are, in addition, fibrinoid masses with blood including detached, sequestrated (necrotic) slices of cartilage. The perichondrium shows slight proliferation, blending with shreds of adherent fibrinoid exudate on the surface. The entire synovial surface exhibits these inflammatory changes.

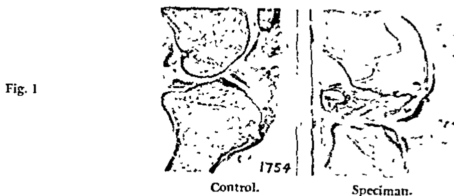


Fig. 1

Control. Speciman.

1800. Third week. The joint cartilage is replaced by firmly adherent granulations, rich in leukocytes and erythrocytes, blending with the subchondral epiphyseal marrow; there is distinct resorption of subchondral bone by dense granulation tissue and marked cellularity of the bone

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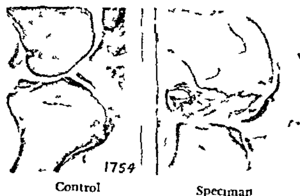


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1800 Third week The joint cartilage is replaced by firmly adherent granulations rich in leukocytes and erythrocytes blending with the subchondral epiphyseal marrow there is distinct resorption of subchondral bone by dense granulation tissue and marked cellularity of the bone

marrow There is very marked periosteal new formation of bone, outside the capsular surface, in part connecting with the marked inflammatory changes of the synovia These include organized fibrinoid exudate, blend ing with the replaced perichondrium and with the underlying chronically inflamed marrow

1773. Fourth week. There is only slight fibrinoid inflammation, partly organized, on the cartilaginous surface The epiphyseal cartilage is as well preserved as in the specimen taken at the first week On the surface of the condyle, there are only shallow erosions in the cartilage

1785. Sixth week. The cartilaginous surface is preserved but markedly vascularized with fibrosing marrow, growing in from the subchondral bone, there is moderate bone and cartilage resorption by giant cells There is little fibrinoid exudate on the joint surface but an increase in fat marrow within the epiphysis

1733. Two months. (Fig 2) The epiphysial cartilage is covered with organized fibrinoid exudate The inner layer of cartilage is well preserved in some areas, in others, surface granulations are connecting with sub chondral bone marrow, causing some osteoclasis There is focal replace ment of epiphysial bone by fibrous marrow, with numerous osteoclasts There is wedge shaped replacement of epiphysial bone by firm organization tissue of callus like appearance bordered, on the surface, by hypertrophic cartilage Fibrosis and edema are noted in the marrow nearby and there is slight osteomyelitic (leukocytic) reaction

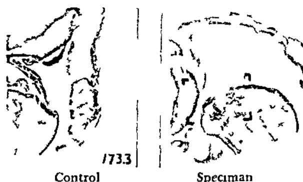
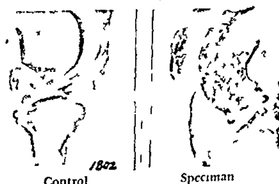


Fig 2

1803. Four months. We note well preserved joint cartilage with lacerated ground substance attached and only slight proliferation of surface cells

1802. Five months. (Fig 3) The joint surface is irregular with deep ulcerations replaced by granulomatous tissue There are fibrosing osteo chondritic changes and massive granulomas within the synovia The sub

Fig 3



chondral bone marrow is fibrous or very cellular, containing large plasma cells and histiocytes, pointing to chronic reactive osteomyelitis. Where cartilage is preserved, it is partly fibrous and shows irregular proliferation. In some areas granulomatous bone marrow faces the joint cavity directly. The surrounding epiphysial trabeculi are thickened and contains fibrous marrow.

1714. **Seven months.** The changes are less marked than those of the fifth month. The surface is largely covered by thickened proliferating cartilage with small polypous protrusions, blending with firm collagenous fibrous tissue including some osteoid bone and continuing into fibrous granulating marrow, in which the newly formed bone shows irregular thickening, but still some osteoclastic activity. There is a distinct increase in the thickness of the bony trabeculi towards the diaphysis with fat marrow. Granuloma formation is less marked within the chronically inflamed fibrous synovia.

1690. **Eighth month.** (Fig 4) The cartilage is preserved, except for a few small erosions containing proliferating cartilage and collagenous fibers. There is distinct focal fibrosis with the underlying epiphysis, with irregular new formation of bone and minimal bone resorption. Some degree of granulomatous synovitis is extending into the epiphysial bone, including the superficial marrow.

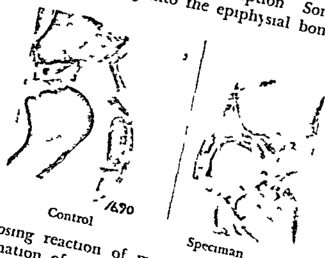


Fig 4

1827. **One month.** Marked fibrosing reaction of marrow, with some edema, and with massive new formation of rather dense bone.

1815. **One month.** Early fibrosing reaction, apparently in connection with the periosteum, surrounding small bony sequestra.

CONCLUSIONS

Definite demonstrable changes occur in joints including the synovia, cartilage and subchondral bone when acrylic is allowed to "cure" *in situ*.

The changes are those of non specific synovial arthritis with osteochondritis, focal osteomyelitic reaction, with subchondral osteoclastic bone resorption and distinct new formation of bone.

On the basis of the material thus far examined, the changes in joints and surrounding bone reach their peak at about the fifth month and show some evidence of subsiding by the eighth month.

†We are indebted to Dr Peter Casagrande Associate in Ortophedic Surgery for assistance and advice to Dr Wallace B Hamby, Prof of Neurological Surgery University of Buffalo School of Medicine for many helpful suggestions and criticisms

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TRANSPLANTABLE OSTEOGENIC SARCOMA OF THE MOUSE EXPOSED TO VITAMIN A INTOXICATION AND VITAMIN D DEFICIENCY*

EDWIN G BOVILL JR

It is not known whether any relationship exists on a general biologic level between epiphyseal cartilage and osteogenic sarcoma beyond their probable common origin from primitive mesoderm There are however certain histologic sequences which are common to each they both yield an increase in tissue mass with the passage of time and they both can produce bone

It is known that vitamin A intoxication and vitamin D deficiency produce predictable gross and microscopic changes in epiphyseal cartilage and growing bone

For these reasons observations of the effect of vitamin A intoxication and of vitamin D deficiency on osteogenic sarcoma were undertaken

METHOD

A review of the compounds listed in the *Negative Cancer Chemotherapy Data* recorded through 1955 reveals no evidence of previous similar observation of these compounds [1]

Lasnitzki has recorded observations on the influence of hypervitaminosis A on the effect of 20 methylcholanthien on mouse prostate glands grown *in vitro* [2] Roncallo and Vascone[3] have reported observations on the influence of hypervitaminosis A on neoplasia caused by benzpyrene The theoretical background for these studies was different from that under consideration in this report since the tumors were not spontaneous and not osteogenic

This osteogenic sarcoma arose at the distal end of the femur in a strain C3Hb/HeN female mouse 34½ months of age which had received no experimental treatment The general behavior of the tumor has corresponded to that previously reported [4, 5] The tumor was obtained from the National Cancer Institute in August 1956 and is still being maintained at the present time Under our observation the tumor has produced death in 45 to 60 days Metastases to lung and liver are common Bone formation is present but minimal

*From the Stanford Medical School San Francisco California Supported in part by a grant from the National Institute of Health U S Public Health Service (C2991)

Fig. 1. Standard Diet

(a)

GUARANTEED ANALYSIS

Crude Protein (minimum)	21.00%
Crude Fat (minimum)	4.00%
Crude Fiber (maximum)	6.00%

5000 U.S.P. Units Vitamin A per lb. of finished feed

INGREDIENTS

Soybean Oil Meal, Cane Molasses, Fish Meal, Condensed Buttermilk, Corn Gluten Meal, Irradiated Brewers' Type Yeast, 4 oz. per ton Wheat Germ Oil, O.P. Linseed Oil Meal, Corn Oil Meal, Ground Oats, Wheat Bran, Wheat Flour Middlings, Ground Yellow Corn, Ground Hulled Barley, Ground Hulled Oats, Ground Whole Wheat, Whole Milk Powder, Alfalfa Leaf Meal, Vitamin A Oil, *½% Steamed Bone Meal, 1% Calcium Carbonate from Limestone, 2% Salt*

(b)

MINERAL COMPOSITION

by chemical determination (per cent)

Silica (Si O 2)	0.59	Calcium (Ca)	2.13
Chlorine (Cl)	0.96	Phosphorus (P)	0.71
Sodium (Na)	0.88	Sulphur (S)	0.24
Potassium (K)	1.12	Magnesium (Mg)	0.33

milligrams per 100 grams

Manganese (Mn)	6.80	Boron (B)	1.37
Iron (Fe)	37.00	Cobalt (Co)	.035
Zinc (Zn)	2.00	Iodine (I)	1.20
Copper (Cu)	0.27	Fluorine (F)	1.90

VITAMIN CONTENT

by rat bioassay determination (per 100 grams)

Vitamin A	610-680 Int'l Units
Thiamine B 1	228-465 micrograms
Riboflavin B 2	538-652 micrograms
Pyridoxine B 3	209-275 micrograms
Pantothenic Acid	1989-2568 micrograms
Choline Equiv.	480-492 milligrams
Inositol (free)†	61 milligrams
Vitamin D	148 U.S.P. Units
Alpha Tocopherol	7 milligrams
PABA‡	78 micrograms

by chemical determination (per 100 grams)

Niacin	14.4 milligrams
Vitamin C	.360 milligrams
Carotene	42-45 milligrams
Choline	152 milligrams

RATIOS

Ca:P		3:1
Ca:Mg		6.5:1
Mn:B 1		14:1
Na:K		1:1.3
anti alopecia	Purified	Stock
inositol diet level	70%	30%

†Mouse bioassay, also by microbiological assay

Prior to subjecting the tumor to the conditions of this experiment it was transplanted through seven successive passages by trocar implantation into the right axilla The host was the C3H mouse obtained from the *Cancer Genetics Laboratory of the University of California* at Berkeley

The standard diet used for all animals except the vitamin D deficient group is shown in Figure 1 The vitamin D deficient diet was identical except for the absence of vitamin D This regime would correspond to the preliminary diet phase used in vitamin D assay studies Early animal mortality has to date interfered with carrying these observations on into the rachitogenic diet phase

Vitamin A in oil was administered parenterally in an equivalent dosage of 250 international units per gram per day, given once weekly Larger doses have been tried and found to result in death of the animals before significant tumor growth was present to record The oil vehicle of vitamin A was also administered to a separate control group

Six to 8 week old animals were used The diets were standard until transplantation of the tumor Administration of vitamin A in excess and the change to the vitamin D deficient diet were respectively carried out at the time of transplantation The animals were sacrificed at 4 to 5 weeks and the weight changes recorded In the vitamin A intoxication group the control animals and tumors weighed slightly more than the treated animals and tumors but the difference was not considered significant (Table 1) *In the vitamin D deficient group* the control and treated

Table 1 Vitamin A Intoxication
Initial Animal Wts 20 gms ± 3

AN NO	CONTROLS AN WT	CONTROLS TUMOR WT	OIL AN WT	OIL TUMOR WT	VITAMIN A AN WT	VITAMIN A TUMOR WT
1	22	2	28	2	18	1½
2	22	7	25	½—	14	½—
3	25	2	22	½—	17	6
4	26	½—	23	3	18	1
5	25	2	22	1	17	2
6	22	2	22	3½	22	5½
7	21	3	23	1	21	1
8	23	4	25	3	17	½
9	21	2½	23	2	16	1
10	23	1	died 1st week		16	1
11	died 1st week		died 1st week		21	1
12	died 1st week		died 1st week		20	1
13	died 1st week		died 1st week		died 1st week	
14	died 1st week		died 1st week		died 1st week	

Table 2 Vitamin D Deficiency
Initial Animal Wts 16 gms ± 2

AN NO	CONTROLS FINAL WT	CONTROLS TUMOR WT	VIT D DEFICIENT DIET FINAL WT	VIT D DEFICIENT DIET TUMOR WT
1	16	1	20	1
2	20	1	died 1st week	
3	25	2	19	½—
4	22	1	23	½
5	19	2	21	½—
6	16	½	20	1
7	21	1	19	½—
8	died 1st week		20	½
9	died 1st week		22	½—
10	died 1st week			

animal weights were the same The tumor weights in the D deficient animals were less than in the controls (Table 2) The autonomous nature of the sarcoma was unaffected The histologic appearance of the sarcoma in the treated animals did not differ from its appearance in the controls either in the soft tissue portions of the tumors or in the areas demonstrating bone formation

CONCLUSION

It is concluded that the typical dependence of epiphyseal cartilage on normal amounts of vitamin A and vitamin D is not reflected in the growing cells of this osteogenic sarcoma under the conditions described

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CATABOLIC EFFECT OF CORTISONE AND ANABOLIC ACTION OF NILEVAR (17 ETHYL 19 NORTESTOSTERONE) STUDIED BY THE RADIOSULFUR UPTAKE IN THE FRACTURED BONES*

K Kowalewski and R K Lyon

The catabolic or anabolic effects of various hormones may be observed in certain clinical syndromes or as the result of therapy In the presence of spontaneous or surgical tissue injury the resulting catabolic response may be aggravated by hormonal imbalance or aided by the hormones promoting anabolism Cortisone is well known as a catabolic steroid inhibiting both chemical and cellular processes associated with inflammation repair and particularly with the synthesis of ground substance The potent components of ground substance are sulfuric mucopolysaccharides combined with proteins and these play an essential role in collagen fibrillogenesis[1]

Using the radiosulfur uptake method the alterations in synthesis of mucopolysaccharides may be well demonstrated This method gives information regarding the location of the tissue sulfates Radiosulfur shows a particular predilection for certain elements of collagen and is selectively incorporated into the cartilage in organic form as chondroitin sulfuric acid[2] It is apparent that the S^{35} uptake technique permits an evaluation of catabolic and anabolic effect of certain hormones on the synthesis of mucopolysaccharides[4, 5]

In our previous report we studied the influence of a synthetic anabolic steroid 17-ethyl 19 nortestosterone (Nilevar) on the S^{35} uptake of fractured humeri in rats We were able to show that Nilevar given to normal or castrated rats during the period of healing of fractures stimulated the S^{35} uptake in fractured bones It was interpreted that Nilevar had marked effects on the synthesis of chondroitin sulfate in the collagen tissues[4]

The purpose of the present experiment was to compare the effects of cortisone and Nilevar on the S^{35} uptake by healing bone fractures An attempt was also made to offset the catabolic effect of cortisone by simultaneous administration of Nilevar to the animals

METHOD

Male rats of the Wistar strain varying in weight from 210 to 320 gm and fed with commercial dog chow were used in the present study

A closed complete fracture of the right humerus was produced in every rat under ether anesthesia

For this purpose the foreleg was grasped between the fingers and thumb of each hand and the bone fractured at its midpoint The animals were kept in separate cages and sacrificed 3 weeks following the fracture

Radiosulfur was given in doses of 200 microcuries by intraperitoneal injection 24 hours prior to sacrifice The dose was dissolved in 2 ml of distilled water together with 8 mg of sodium sulfate

Four groups of rats were studied The first group received no treatment and served as a control The second group of animals was treated with

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*From the McEachern Cancer Research Laboratory and Department of Surgery University of Alberta Edmonton Canada Supported in part by a grant from Searle & Co Chicago Ill who also furnished crystalline Nilevar

cortisone administered subcutaneously in doses of 10 mg dissolved in 0 5 ml of saline Each animal received 10 doses, the total dose being 100 mg per rat The animals of the third group received both cortisone and Nilevar treatment Cortisone was given in the same way as for group 2 Nilevar was injected intramuscularly in 10 doses of 5 mg each, dissolved in 0 4 ml of sesame oil, the total dose per rat being 50 mg The last group of rats was treated with Nilevar alone, and the total dose per animal was also 50 mg The injections were given 3 or 4 times weekly during the 3 week period of healing, 4 injections the first week and 3 injections weekly for the next 2 weeks

The processing of bones for the final counting of radioactive barium sulfate and the method of counting were exactly the same as described in detail in our previous report[6] The results were expressed in counts per minute (c p m)/100 mg of tissue and F/I ratio was calculated This is the ratio of the uptake (c p m /100 mg of bone) of S^{35} by the fractured humerus (F) to the uptake of S^{35} by the corresponding intact bone (I)

RESULTS

The results are summarized in Figure 1 It may be noted that there is a significant reduction of F/I ratio in rats treated with cortisone as compared with normal control animals

Simultaneous treatment with cortisone and Nilevar markedly offsets this effect of cortisone Nilevar alone promotes a significant increase of F/I ratio

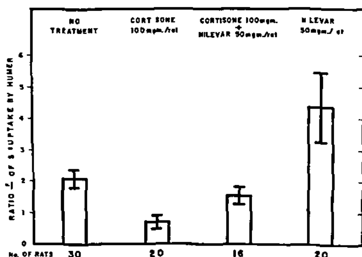


Fig 1 Effect of cortisone and nilevar on S^{35} uptake by fractured (F) and intact (I) humerus expressed as F/I ratio Mean values and S D (vertical lines).

COMMENT

The inhibitory effect of cortisone on tissue repair, demonstrated by the other techniques was also proved in our experimental conditions Because cortisone blocks the production of mucopolysaccharides which specifically incorporates radioactive sulfur, the proposed uptake method is very adequate to study the effect of this hormone

This method shows that the synthetic androgen Nilevar is a highly stimulating factor of bone healing, which probably means that Nilevar promotes the synthesis of collagen during the process of repair. Combined therapy protects connective tissue against the effect of cortisone.

SUMMARY

1. The study of the uptake of radiosulfur in the intact and fractured humeri of rats was performed and the ratio F/I of radioactivity of fractured (F) to intact (I) bone was calculated.

2. The effect of treatment with cortisone and Nilevar (17 ethyl-19 nortestosterone) on the S^{35} uptake in the fractured humeri was studied

3 Cortisone treatment results in significant decrease of F/I ratio, as compared with normal control.

4. Nilevar stimulates the S^{35} uptake in fractured bones resulting in high F/I ratio

5 Combined therapy with both hormones partially offsets the effect of cortisone.

6. It is apparent from this study that cortisone inhibits and Nilevar promotes those processes of repair which may be measured by S^{35} uptake method, and that Nilevar may protect connective tissue against the catabolic effect of cortisone

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THE EFFECT OF TOTAL BODY IRRADIATION ON BONE TRANSPLANTS IN PARABIOSED ANIMALS*

W F Enneking, Avrum Gratch, and Heber Ethridge

Previous studies in unselected female rats have shown that large fresh homogenous bone transplants evoked a low grade inflammatory response in the host tissues about the transplant

Fresh homogenous bone transplantation was repeated employing the same donor and recipient animals In those animals which had produced an inflammatory response to the first transplant, the second transplant evoked a more violent inflammatory response

Animals were surgically parabiosed and large fresh homogenous bone transplants exchanged between members of the union An acute inflam matory response sequestrated the transplant in over 80% of the animals Although one cannot precisely quantitate histologic interpretation, a graph ic summary of this work shows the relationships in a general fashion between homogenous, presensitized homogenous, parabiotic, and control autogenous transplants

The experiments being reported were done to determine the effect of suppressing antibody production while exchanging homogenous bone trans plants in parabiosed rats It was felt that if one member of a parabiotic union received total body irradiation while the other member was shielded from the x rays, a situation would exist in which the nonirradiated animal would be incapable of antibody production If a relationship exists between circulating antibodies and the accelerated inflammatory response to homogenous bone transplants there might be a detectable difference in the host response to transplants in this experimental situation The irradiated animal, being incapable of antibody production, should show a diminished inflammatory response to the homogenous transplant The nonirradiated animal, being able to form antibodies and acting as the control, should show the previously described accelerated inflammatory response to the homogenous transplant

The variables in this experiment were the amount of depression of the immunological mechanism produced by the dose of irradiation, the degree of incompatibility of the animals selected as partners for parabiosis, and the effect of radiation upon bone repair The varying amount of depres sion of the immunological mechanism was combatted by heavy and reg ularly repeated doses of radiation

The incidence and site of fatal parabiotic intoxication furnished a control for the effectiveness of the dosage

Randomly selected animals were used as partners to minimize the occur rence of mutually compatible or isogenic pairs The animals were chal lenged by skin transplants after completion of the bone transplants for compatibility

Comparison of the nonirradiated and the irradiated animals served as a control in evaluating the effect of radiation upon bone repair

*From the University of Chicago Medical Clinics and the University of Mississippi Medical Center Supported by P H S Grant No 1467

METHOD

Female Sprague Dawley rats weighing 100 gm received total body irradiation of 100r

These animals were then surgically parabiosed with nonirradiated rats of the same size and sex 48 to 72 hours following their initial irradiation The dose of total body irradiation was repeated at 7 day intervals through out the course of the experiment to the irradiated member of the union while shielding the nonirradiated animal

Full circumference sections of the femoral shafts with attached periosteum were exchanged and immobilized 24 to 35 days after parabiosis Full length sagittal sections of the femur were obtained and prepared for histologic study

The parabiosed animals were maintained for 6 months or longer with out radiation following completion of the bone experiments They were then surgically separated in such a fashion that a broad based pedicle of skin and subcutaneous tissue from 1 animal was incorporated as a portion of the integument of its partner

RESULTS

A total of 373 parabiotic unions was prepared One or more members of 81 unions died of shock or overdose of anesthesia Sixty-one unions had one or both members die from infection, evisceration, or trauma Of the remaining 231 unions, 99 had one member die of parabiotic intoxi cation between the tenth and twenty first post union days In all but 3 of these unions death occurred in the irradiated member The incidence then of fatal parabiotic intoxication in those animals who survived 10 days was 43% Ten unions died after 21 post union days of infection or unknown causes Thus of the original 373 unions 118 survived and had bone transplants exchanged between them Of these animals 104 prepara tions from 52 pairs were studied

There was no detectable difference in the healing of the clean wounds produced by surgical parabiosis or transplantation in the irradiated animals as compared to the nonirradiated animals There was however, a signifi cant increase in the incidence of indolent wound infections in the irradiated animals

Histologic studies of the preparations obtained prior to 7 days after transplantation showed no significant difference in response between the irradiated and nonirradiated animals

The preparations from the 47 unions sacrificed after the 7th day and up to the 120th day showed two divergent circumstances

In 16 of the unions the irradiated animal did not produce an inflam matory response to the transplant while the nonirradiated animal did produce an inflammatory response which was sufficient to sequestrate the transplant from the repairative processes

In 28 of the unions neither the irradiated animal nor the nonirradiated animal produced an inflammatory response to the transplant

There were 3 unions in which the irradiated animal produced an inflammatory response

Twenty four of the above 47 pairs survived 6 months after bilateral hip

disarticulation Separation with skin transplantation was accomplished in these animals Fourteen animals died as an immediate result of the procedure Five animals showed necrosis of the transplanted skin within 48 hours of transfer Four animals from 2 pairs accepted the skin from their partners with survival of the skin to date or to their demise

Twenty five animals showed survival of the transplanted skin for a period ranging from 6 to 12 days after separation Inflammation then developed about the transplants and necrosis followed in 3 to 5 more days Biopsies of these skin transplants made 3 to 5 days following separation and prior to the onset of necrosis showed patent vascular supply with no deep necrosis

SUMMARY

Total body irradiation appears to be effective in blocking the inflammatory response to large fresh homogenous bone transplants in parabiosed rats

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A STUDY OF BACK PAIN THE USE OF PROGRESSIVE RESISTANCE EXERCISES AS A PRIMARY MODE OF TREATMENT*

MARVIN H DUBANSKY AND CARROLL B LARSON

This project is a pilot study of low back pain An attempt is made to classify types of backache on a clinical, radiographic and pathologic basis Although some cases were seen during the acute phase, the majority were of the chronic recurrent variety A complete physical examination, including all information vital to the history and physical examination of the back was followed A routine set of anterioposterior, flexion and extension lateral and standing lateral x ray films were taken on all patients In some cases oblique views were also obtained if indicated All examinations and x ray interpretations were made by the authors The acute backaches were treated by rest, heat, massage, and supportive care as indicated All chronic cases and the acute cases, while in the quiescent or chronic phase, were treated by a set standard of exercises performed under the direction of the authors No exercises were undertaken until the patient could perform them without pain These exercises were as follows 1 A sit up with the knees and hips flexed, the hands in back of the neck By means of a special harness and table, weight was progressively added for resistance 2 Back extension The patient lay prone on the table flexed over the edge with the legs stabilized He then placed

*From the State University of Iowa Aided by grant from the Athletic Department State University of Iowa

the hands on the back of the neck and raised the trunk level with the table A similar type of weight resistance apparatus was used

The diagnosis and criteria for same were as follows

Instability. Excessive or asynchronous motion of one vertebral body upon the adjacent one by x ray This was correlated with the clinical finding of an 'Instability test' performed by having the patient completely relaxed in a prone position Diffuse pressure was applied in the area of pain with the heel of the hand If the patient had pain relieved by active extension of the trunk, the test was considered positive If the pain was not relieved with active extension, the test was considered negative

Degenerated Disc. Although it has been considered that the presence of instability, osteophytic formation, and the clinical syndrome of a herniated nucleus pulposis are due to degeneration, for the basis of this paper, only those interspaces which were markedly narrowed relative to the other interspaces was such a diagnosis made

Herniated Nucleus Pulposis These patients had radicular pain to, at least, the back of the knee, a restricted straight leg raising test, and/or sensory or reflex changes consistent with such a diagnosis

Facet Syndrome A definite history of 'catches with x ray evidence of congenital facet changes

Low Back Pain. There was a moderate sized group who could not otherwise be classified Clinical and radiographic studies did not help separate these into clinical categories They were, therefore, classified as low back pain with or without congenital abnormalities of the spine

All subjects were considered thus to have mechanical backache Any subjects thought to have backache due to other causes were excluded

Forty five subjects were examined and treated as outlined Of this group 12 were considered to have degeneration, 8, instability, 4, degeneration and instability, 6, low back pain with anomalies, 12, low back pain without anomalies There was one case each of facet syndrome and ruptured disc Nineteen controls were x rayed and underwent 1 and 10 lift flexion and extension maximum tests

Of the 45 subjects, only 2 were not relieved of their symptoms One patient was unstable and the other was considered to be low back pain The average strength figures for the control subjects were distinctly higher than the initial lift of the patients However, at the termination of the exercise periods, the patients' average strength was in excess of the controls' The 2 patients who were not subjectively relieved of their symptoms did not obtain strength levels of the controls The average number of exercise periods was 26 An attempt was made to break down the figures for each clinical entity, but due to the small number of cases, definite conclusions could not be drawn, except that patients with disc degeneration were distinctly weaker before and after exercise than all other groups

DISCUSSION

Strength of abdominal and back musculature was studied and compared with 19 control subjects It was found that the average strength of the patients was 23 pounds less for back strength and 10 pounds less for abdominal strength than that of the controls At the termination of the

Table 1 One Lift and Ten Lifts Maximums Before and After Exercise

	BEFORE EXERCISE MEAN	AFTER EXERCISE MEAN	DIFFERENCE IN POUNDS	INCREASE IN PERCENT
Back Extension (1) *	65 83	99.34	33 66	51 13
Back Extension (10)	46 71	72 00	25.29	54 14
Leg Extension (1)	60 80	91.27	30 47	50 12
Leg Extension (10)	42 90	68 65	25 75	60 02
Trunk Flexion (1)	19 46	32 86	13 40	68 86
Trunk Flexion (10)	12 98	24 16	11 18	86 13

*The numbers in parentheses indicate whether the test was for a one lift maximum or ten lift maximum

exercise period, the average back strength was 10 pounds higher and abdominal strength was 3 5 pounds higher than that of the controls

Greatest gain in pounds was recorded for the back extension movement which was approximately 3 times that recorded for trunk flexion The greatest gain in percentage based on initial strength recorded was made, however, in trunk flexion It is evident that the back muscles are stronger than the abdominal muscles, for the exercises performed in this study, in the order of a 3 to 1 ratio

Table 2

RATIO	INITIAL FREQUENCY	FINAL FREQUENCY
17 17 99	3	0
16 16 99	0	0
15	0	0
14	0	0
13	0	0
12	1	0
11	0	0
10	1	0
9	0	0
8	0	1
7	0	0
6	1	0
5	3	4
4	11	8
3	17	14
2	14	29
1	7	2

A study of 16 patients with no back symptoms was used as the control The mean back strength of this group was 98 25, and the mean abdominal strength was 34 30 These figures were very close to the means found for the experimental group of 45 subjects after they had concluded their exercises The mean back/abdominal ratio of 2 88 was slightly lower than that of 3 24 for the experimental group

It was also interesting to note the frequency of the ratios at the beginning and end of the experiment period as shown in the following table

The mode of the final distribution is between 2 and 2 999, with the final distribution more homogenous than the initial distribution

From this initial data it would appear that a normal ratio of back to abdominal strength exists, this being in the range of 3 1 If this ratio is exceeded, the possibilities of mechanical backache are excellent and reversal is desired for a control of symptoms

For further study, more prolonged followup is necessary Additional cases will insure a better statistical study of the various groups

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EXPERIMENTAL EPIPHYSIOLYSIS IN RATS*

J WILLIAM HILLMAN, WILLIAM A HUNTER, JR AND JOHN A BARROW, III

The epiphyseal growth zone of long bones offers a medium for demonstration of certain physiologic responses of the immature laboratory animal The present report is concerned with observations on the tensile properties of the upper tibial epiphysis of the immature rat using the technique described by Harris[1] This method has been used to measure in a quantitative manner the healing responses of the epiphyseal growth zone following local injury (experimental epiphysiolysis) both in normal animals and in animals where estradiol benzoate was administered

METHOD

Sprague Dawley rats weighing 40 60 gm (24 26 days of age) were used with an equal number of males and females in each series The diet was Purina Rat Checkers and water fed *ad libitum* All animals gained weight appropriately and there was no sign of illness in the colony during the test periods

The laboratory equipment used to determine the force required to displace the epiphysis from the metaphysis of the upper tibia, as described by Harris, is illustrated in Figure 1 The weight which caused the separation is recorded as the *shearing force required* Modifications of the test equipment were largely concerned with the selection of needles strong enough

*From the Division of Orthopedic Surgery, Department of Surgery Vanderbilt University School of Medicine Nashville Tennessee

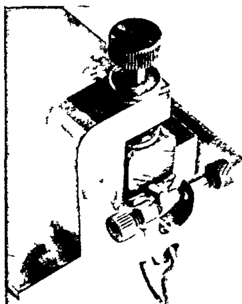


Fig 1 Modified Harris apparatus The upper tibial ossification center is grasped by a metal Y shaped yoke through which screws are threaded with needle points in their central axis After this device is centered and attached with care to avoid damage to the growth cartilage the metaphysis is clamped in the vise Wooden blocks with carved indentations stabilize the shaft without crushing Beneath the Y yoke is suspended a receptacle to receive sand from a reservoir As sand is slowly allowed to fall an abrupt end point is determined when the epiphysis becomes separated

to tolerate the forces applied yet small enough in diameter to avoid premature separation of the epiphysis by a wedging effect At the present time milliners needles with diameter of 0 5 mm and exposed length of 3 0 mm are used

In preparation of the tibia for testing the leg is amputated and dissection is carried out in subperiosteal fashion removing all investing soft tissue at the epiphyseal line with care to avoid damage to the periphery of the cartilage plate or adjacent bone The bone is mounted horizontally with the anterior surface down since this position offers less possibility that remaining elements of the patellar tendon insertion might interfere with measurement of the cartilage strength

Standardization of the error inherent in the Harris test was carried out by the use of approximately 50 animals with equal number of males and females weighing between 50 gm and 250 gm In the smaller weight ranges the standard error was found to be 65 gm while in the animals 250 gm and over the forces required were so large that erratic results were encountered occasionally with fracture of the diaphysis before lysis occurred For this reason the test animals in these experiments were less than 250 gm body weight

Surgical epiphysiolysis is performed through an incision 1 cm in length over the anteromedial aspect of the knee joint with anesthesia obtained by subcutaneous injection of pentobarbital The periosteum over the epiphyseal growth line is incised transversely for a distance sufficient to allow insertion of a small scalpel blade or flat needle into the cartilage The blade is used as a lever and by manipulation of the leg the epiphysis loosened sufficiently to insure adequate displacement The incision is closed with 3 #80 nylon subcuticular sutures and collodion The animal is allowed to move freely after recovery from anesthesia

Experiment A Definition of weight strength relationship in normal animals The initial experiment compares the relationship between the weight of the animal and grams force required to shear the upper tibial epiphysis

Twenty six male and 26 female rats were tested as male and female pairs in serial fashion at 48 hour intervals

Experiment B Influence of estradiol benzoate administration on epiphyseal strength Twenty rats weighing from 40 50 gm were tested in a manner entirely similar to Experiment A with the exception that 100 gamma estradiol benzoate was given by intramuscular injection every 48 hours beginning 8 days prior to the test period and continuing throughout the period of serial testing

Experiment C Recovery of epiphyseal cartilage strength following experimental epiphysiolysis in normal animals Using the technique described above, surgical epiphysiolysis was performed on the right leg of 8 rats weighing 80 100 gm Control values were obtained from study of an equal number of litter mates Animals were sacrificed serially beginning at 48 hours and extending to the ninth day

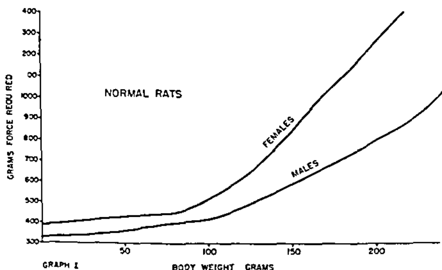
Experiment D Recovery of epiphyseal cartilage strength following combined experimental epiphysiolysis and estradiol benzoate administration This group of 8 rats was studied by combining the administration of estradiol benzoate as described in Experiment B with lysis of the tibial epiphysis as outlined in Experiment C with similar serial testing of strength in the epiphysis

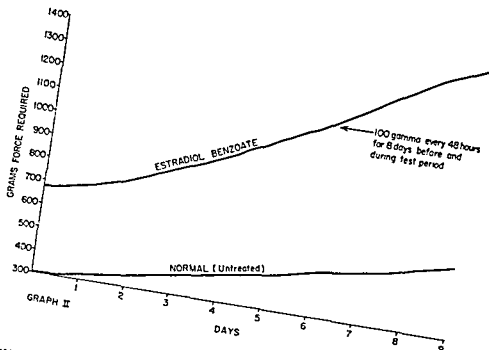
RESULTS

The increase in body weight of normal immature rats is accompanied by a progressive increase in epiphyseal strength Figure 2A illustrates this relationship through the period of growth between 50 gm initial weight and a final weight of 150 250 gm While the body weight of the female animals was slightly less than the male animals the force required for displacement was consistently greater in the females

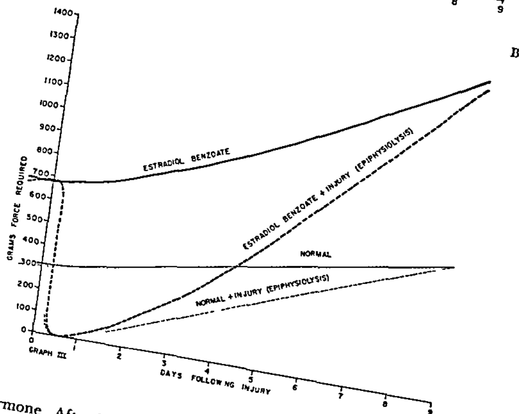
Figure 2B shows that the administration of estradiol benzoate significantly increases the inherent strength of the intact epiphysis and that this effect may be sustained through a period of growth by continued administration

Fig 2





B



C

of the hormone After 18 days administration, the values recorded indicated more than doubling of the epiphyseal strength when compared with untreated animals of the same age.

Experimental disruption of continuity of the epiphysis in 80-90 gm animals as shown in Figure 2C initiated a vigorous healing reaction which gained strength steadily until the ninth postoperative day at which time the tibiae which had been injured required the same force for displacement as the unoperated controls A striking similarity was found in the time requirements for the restoration of strength in these animals when compared

Twenty six male and 26 female rats were tested as male and female pairs in serial fashion at 48 hour intervals

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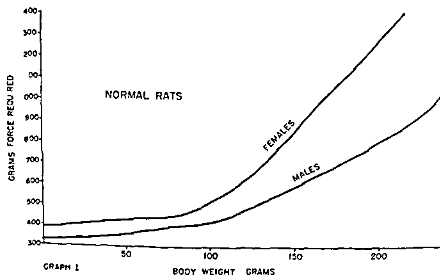
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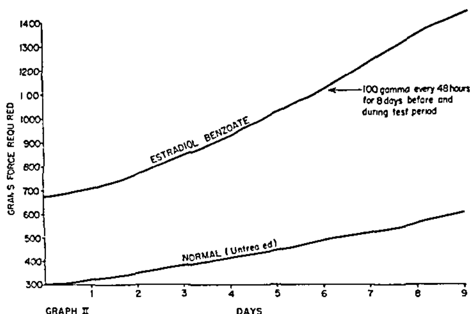
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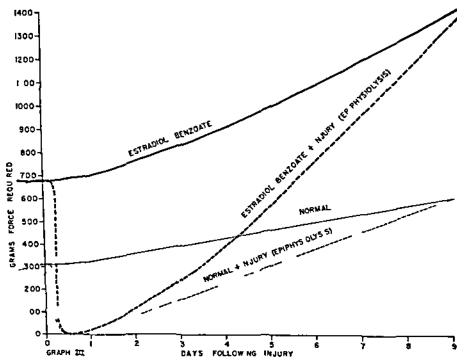
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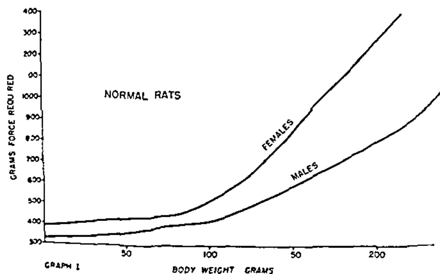
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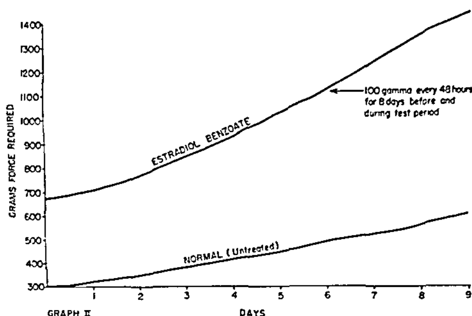
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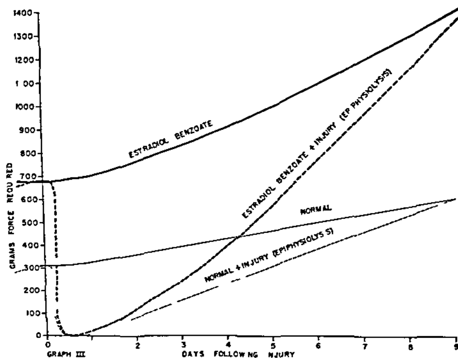
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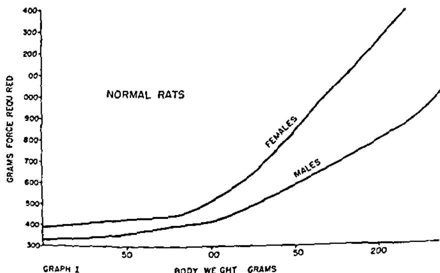
Experiment D Recovery of epiphyseal cartilage strength following combined experimental epiphysiolysis and estradiol benzoate administration This group of 8 rats was studied by combining the administration of estradiol benzoate as described in Experiment B with lysis of the tibial epiphysis as outlined in Experiment C with similar serial testing of strength in the epiphysis

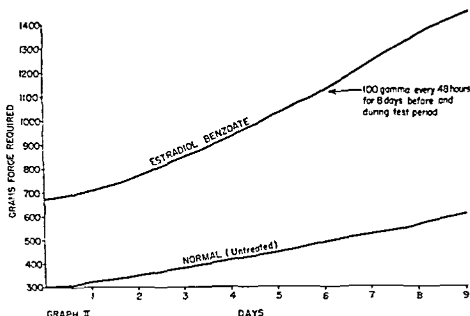
RESULTS

The increase in body weight of normal immature rats is accompanied by a progressive increase in epiphyseal strength Figure 2A illustrates this relationship through the period of growth between 50 gm initial weight and a final weight of 150 250 gm While the body weight of the female animals was slightly less than the male animals the force required for displacement was consistently greater in the females

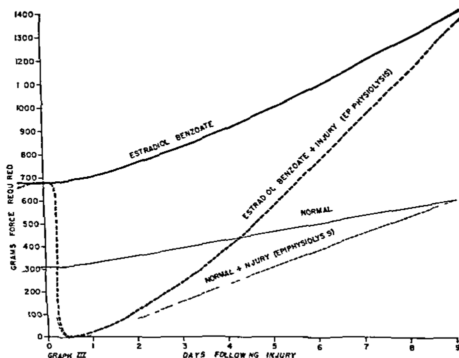
Figure 2B shows that the administration of estradiol benzoate significantly increases the inherent strength of the intact epiphysis and that this effect may be sustained through a period of growth by continued administration

Fig 2





B



C

of the hormone After 18 days administration, the values recorded indicated more than doubling of the epiphyseal strength when compared with untreated animals of the same age

Experimental disruption of continuity of the epiphysis in 80-90 gm animals as shown in Figure 2C initiated a vigorous healing reaction which gained strength steadily until the ninth postoperative day at which time the tibiae which had been injured required the same force for displacement as the unoperated controls A striking similarity was found in the time requirements for the restoration of strength in these animals when compared

with the series which had been treated with estradiol benzoate since 9 days had elapsed in each case before tissue strength at the epiphyseal plate had become equivalent to the tissue strength of the control animals. The contrast between the two groups was apparent, therefore, not in time relationships, but in the tensile strength values at the time the injured bones rejoined their controls, i.e., on the ninth day in both series. The estrogen treated animals required 1300-1400 gm. for displacement while the control animals which received local injury or no interference with normal growth remained in the 600-800 gm. range. These observations would suggest that *estradiol benzoate potentiated the tensile properties of the connective* tissue, both with and without local injury, but was not capable of influencing the rate of maturation and organization of the tissue elements.

DISCUSSION

In the studies presented, intact growing rats were found to demonstrate an alteration of physical characteristics of the epiphyseal growth line when subjected to two test situations, administration of estradiol benzoate and creation of localized injury of the growth plate. The results show a significant degree of response which can be measured quantitatively and can be readily reproduced. The number of animals used was small and no direct clinical implications are offered. It is apparent, however, that further investigations with this approach may prove useful in evaluation of steroid compounds and metabolic factors which affect the epiphyseal zone. The results of these future studies may have clinical significance in the etiology and treatment of slipping of the upper femoral epiphysis, which frequently occurs in children with growth disturbance in the age limits of puberty.

One of the more widely employed techniques in current use for evaluation of steroidal activity consists of determination of myotrophic effect in castrated male rats, as described by Eisenberg and Gordan.[illegible] In that test, the increase in weight of the levator ani muscle is measured after dissection and compared with the muscle weight in control animals. The widely spaced variations in epiphyseal strength demonstrated in the present studies would suggest that this test offers a possibility of studying the "chondrotrophic" effect of certain steroids in the same manner as the Eisenberg-Gordan test demonstrates myotrophic effect. Of course, additional correlation must first be obtained by determination of these and other values for castrated animals and determination of the response to variations of dosage and duration of treatment. The administration of estradiol benzoate for 8 days prior to initiation of testing as described in this report was, for example, arbitrarily chosen to insure an adequate time for an effect, if any, to appear.

SUMMARY

The tensile strength of the upper tibia epiphysis of the rat was measured to demonstrate (a) relationship between increments of growth and strength, (b) the effect of estradiol benzoate administration, (c) the response to local injury (epiphysiolysis), and (d) the response to combined estradiol benzoate administration and local injury.

It is proposed that this test may be developed as a method of bio-assay. Further studies with this technique are indicated to investigate the possible

endocrine relationships in certain clinical conditions, notably slipped upper femoral epiphysis

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THE EFFECT OF THE ADDITION OF PLASTER OF PARIS TO AUTOGENOUS AND HOMOGENOUS BONE GRAFT IN DOGS*

LEONARD F PELTIER AND DUANE ORN

Our interest in using plaster of Paris in reconstructing major defects in long bones was aroused by the paper of Kovacevic[1] who reported upon three patients with extensive hematogenous osteomyelitis of the tibia Following diaphysectomy, the resulting defects in the tibia were filled with plaster of Paris The plaster was slowly absorbed and new bone regenerated, filling in the defects in the diaphysis

The use of plaster of Paris to reconstitute diaphyseal defects in the radius of dogs was investigated and has been reported upon elsewhere[2,3] It was found that the plaster of Paris was slowly absorbed from the area of implantation, and that when endosteal or periosteal tissue was present, regeneration of bone occurred in a portion of the animals

The present report is concerned with the effect of the addition of plaster of Paris to bone grafts used to reconstitute similar diaphyseal defects in the radius of dogs

METHOD

Mongrel dogs over one year of age were used General anesthesia was obtained by the intravenous injection of sodium pentobarbital Operations were carried out with antiseptic technique A tourniquet was used to provide hematosis during the operation The extremity was protected by a light splint for about 3 weeks postoperatively No antibiotic drugs were used Roentgenograms were taken at the end of the operation and at the time of sacrifice of the animals At autopsy the operative site was carefully examined and histologic sections were cut through the area of the graft

A standard defect was made by resecting a portion 4 to 5 cm in length of the shaft of the radius and its enveloping periosteum The resected bone and periosteum were passed through a bone mill and used as a fresh

*From the Division of Orthopedic Surgery University of Minnesota Medical School Minneapolis Supported by a grant from the Graduate School of the University of Minnesota and by U S P H S grant #RG 4985

graft to repair the defects Preformed columns of plaster of Paris, 2x5 cm, in length, sterilized by dry heat, were added to some of the grafts Four different methods were employed to reconstitute the standard defect in the radius

1 *Autogenous graft alone* The resected bone was passed through the mill and the bone chips were replaced in the defect

2 *Autogenous bone plus plaster of Paris* After carrying out the grafting procedure, a column of plaster of Paris was also placed in the defect

3 *Homogenous graft alone* Dogs were operated upon in pairs The resected bone from one dog was passed through the bone mill and the bone chips were then used to fill the defects in the radius of the other dog

4 *Homogenous bone graft plus plaster of Paris* After performing the homogenous graft a column of plaster of Paris was also placed in the defect

RESULTS

In a group of 9 dogs in which resection of 4 to 5 cm of the shaft of the radius and its periosteum was carried out without attempting reconstitution of the defects, there was no spontaneous regeneration of bone in the defects of any of the animals [3] In such a standard defect, therefore, any bony regeneration in the area of the defect must be due to the reconstruction procedure carried out Thirty three dogs were followed to a definite end result, 31 for 90 days or more The amount of new bone formed in the defect was estimated from the comparison of the roentgenograms made at the time of the operation and at the time of sacrifice When it appeared that the amount of bone in the defect was neither greater nor smaller than in the initial roentgenogram, the animal was placed in Group O, i e, no change When there was more bone visible in the final film, the dog was placed in Group 1+, or when complete restoration had occurred in Group 2+ When there was resorption of the bone graft on the final film, the dog was placed in Group 1−, or when absorption was complete, in Group 2− The results of the experiment are shown in Tables 1, 2, 3, and 4

Table 1 Reconstitution Diaphyseal Defects in Radius Autogenous Bone Graft

DOG NO	POSTOPERATIVE FOLLOW UP IN DAYS	GROUP 2+	1+	0	1−	2−
1	101	x				
2	101		x			
3	106	x				
4	103	x				
5	102	x				
6	102	x				
7	103			x		
Totals	7 dogs	5	1	1		

Table 2 Reconstitution Diaphyseal Defect in Radius Autogenous Bone Graft Plus Plaster of Paris

	POSTOPERATIVE FOLLOWUP			GROUP		
DOG NO	IN DAYS	2+	1+	0	1—	2—
8	90			x		
9	105	x				
10	92	x				
11	98	x				
12	96		x			
13	92	x				
Totals	6 dogs	4	1	1		

Table 3 Reconstitution Diaphyseal Defect in Radius Homogenous Bone Graft

	POSTOPERATIVE FOLLOWUP			GROUP		
DOG NO	IN DAYS	2+	1+	0	1—	2—
14	66		x			
15	91	x				
16	95			x		
17	86			x		
18	107				x	
19	102					x
20	102				x	
21	109					x
22	109			x		
Totals	9 dogs	1	1	3	2	2

Table 4 Reconstitution of Diaphyseal Defect in Radius Homogenous Bone Graft Plus Plaster of Paris

	POSTOPERATIVE FOLLOWUP			GROUP		
DOG NO	IN DAYS	2+	1+	0	1—	2—
23	104		x			
24	100	x				
25	111				x	
26	111				x	
27	100					x
28	100					x
29	107	x				
30	107	x				
31	108			x		
32	108					x
33	113					x
Totals	11 dogs	3	1	1	2	4

The histologic sections demonstrated again that plaster of Paris does not produce an inflammatory reaction in the area of implantation

DISCUSSION

The superiority of fresh autogenous bone grafts over fresh homogenous bone grafts is easily demonstrated by comparing Tables 1 and 3 The addition of plaster of Paris to autogenous bone grafts does not appear either to promote or to inhibit the survival of the graft or the regeneration of bone The addition of plaster of Paris to homogenous bone grafts resulted in a slightly greater number of successes and failures than with homogenous bone grafts alone It is doubtful if these variations are significant but it does direct attention to the possibility that success of homogenous grafts may be influenced favorably or unfavorably by additives — a prospect which deserves further investigation The most important finding of this investigation is that the reaction of the tissue to the implantation of large quantities of plaster of Paris in no way prejudices normal osteogenesis

CONCLUSION

Large quantities of plaster of Paris were added to fresh autogenous and homogenous bone grafts used to fill diaphyseal defects in the radius of dogs There was no evidence that the presence of the plaster of Paris affected bone regeneration adversely in any of the dogs The autogenous grafts were essentially uneffected The addition of the plaster of Paris to homogenous bone grafts resulted in a small increase in the number of defects showing full regeneration

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THE EFFECTS OF IRRADIATION UPON BLOOD VESSELS AND THE SURVIVAL OF SKIN AUTOGRAFTS*

SHELDON O BURMAN

During the interval between the transplantation of a skin autograft and its revascularization the metabolic transport of the graft occurs by diffusion through a viscous proteinaceous extracellular fluid present at the interface (Haynes[7]) — the Plasmatische Circulation (Goldman[6]) Following this interim phase a characteristic vascular phenomenon occurs in which knob like tufts grow from the existing capillaries of the base and periphery of the recipient site in a parallel orderly fashion toward the empty, widely patent vessels of the graft (Conway[4] *et al*) Other factors being equal the survival of the autotransplant is directly dependent upon the early re establishment of this circulation between the graft vessels and those of the recipient site (Conway[4] *et al* Edgerton[5] *et al*) To determine if the profound changes wrought by roentgen irradiation upon blood vessels could affect the revascularization and ultimate survival of skin autografts placed upon an irradiated site the following procedure was undertaken

METHOD

Both ears of 10 albino rabbits of mixed stock were shaved and 3 circular pieces of skin approximately 12 mm in diameter were cut from the dorsum of each ear The full thickness of skin was removed care being taken not to damage the subcutaneous vascular plexus The grafts from the left ears were sutured with 5 0 interrupted silk to the recipient sites upon the right ear Those from the right ear were simply laid unsutured upon the recipient site of the left ear their fate being unimportant to our purposes

Since the rabbits make no attempt to dislodge the grafts no dressings or splints were used The absence of dressings permits daily inspection of the grafts During the preliminary phase of this experiment sufficient skill was acquired to ensure that any failure of survival was not attributable to faulty technique At daily intervals following grafting 4 to 6 ml of 4% bromophenol blue were injected over a period of about one minute into the marginal vein of the ear (Scothorne & McGregor[9]) The dye solution was prepared by adding 15 ml of N/5 NaOH to one gram of bromophenol blue and the total made up to 25 ml with 0 9% NaCl solution Normally vascularized tissues are colored intensely blue immediately upon injection the depth of the color being roughly proportional to the degree of vas cularity Relatively avascular tissue becomes colored only after several hours by diffusion The dye is entirely harmless and 24 hours later vas cularized tissues are no longer colored Dye is removed from nonvascular

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ized tissues far more slowly, however, and 24 hours after the injection *nonvascularized skin grafts are still faintly blue* The color changes in the skin of the ear surrounding the grafts serve as controls

After ascertaining the normal revascularization time for this group of nonirradiated control animals, the right ears of 40 albino rabbits of varying ages were given sufficient radiation to produce a severe erythema The dosage varied with the age of the animals, 3500 r being sufficient radiation for the youngest, while the largest and oldest required up to three times as much to produce a severe erythema For each treatment 550 r were delivered, KVP = 60, MA = 20, VHL = 0 5 mm Al, TSD = 16 cm, field size = 15 cm circle Skin grafts were then performed at 2 days and at 1, 2, 3, 4, 6, 8, 10 and 12 weeks after irradiation Grafts have not yet been transplanted at 16 and 24 weeks

RESULTS

On the first day after grafting unirradiated, control animals the grafts remain starkly white while the surrounding skin is colored intensely blue On each succeeding day following the dye injection the grafts become increasingly blue until on the fifth day the color of the graft approximates that of the ear Thus, effective revascularization of a skin autograft in a rabbit's ear occurs between days 4 and 5

Following irradiation, during the initial stage of erythema, revascularization was complete in 3 days As the time interval following irradiation increased, the revascularization time lengthened toward that of the controls and then continued to increase until at 4 weeks, 2 grafts failed while the remainder required 7 days to revascularize At 6 and 8 weeks, 8 days were required but a high percentage of these grafts failed At 10 weeks the percentage of graft survivals increased sharply and those which revascularized did so within 8 days At 12 and 16 weeks there were no failures, and the time required for revascularization decreased progressively towards normal In each case where a graft failed, its color never became more than faintly blue

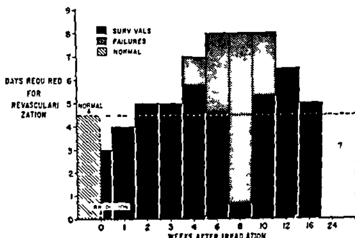


Fig 1

DISCUSSION

Conway and Edgerton, using the transparent chamber technique, demonstrated that successful grafts occur only when blood vessels of the recipient area remain patent and exhibit active circulation. In addition to patency the vessels must exhibit the ability to respond to the presence of a graft by sending budding capillary tufts growing toward it. It is clear that this angioblastic activity occurs both from the base underlying the graft as well as from the periphery of the recipient site (Borak[2]). Also necessary to the survival of the graft are other vascular phenomena, defined by Chambers and Zweifach,[3] such as contraction of the precapillary sphincter, the alternate opening and filling of the capillary bed, and its occlusion by the sphincter preliminary to emptying.

The earliest visible effects of irradiation upon the skin are erythema and edema. During this period, the revascularization time was noted to decrease sharply. The existing capillaries are engorged with blood and endothelial permeability apparently is increased. While these factors should provide an extra measure of security for the graft before revascularization occurs, the rapid ingrowth of new vessels may perhaps be explained by an increase in angioblastic activity of capillary endothelial cells. Maximow[5] believes that this angioblastic process is limited to capillaries since "blood vessels which have muscle and elastic fibres in their walls... are devoid of this capacity."

The inflammatory stage following hyperemia and edema is characterized by the occurrence of arteritis and phlebitis. Added to endothelial swelling and subendothelial exudation this inflammation of the vessel wall contributes further to the narrowing of the lumen, even to complete occlusion with cessation of blood flow. Endothelial cells showing vacuolization and proliferation following irradiation are unable to form new capillaries. This may be considered one of the most important results of irradiation, and the process of repair is suspended until the damaged capillaries recover and regain their angioblastic proclivities (Conway *et al.*[4]). During this phase the revascularization of autografts is greatly jeopardized and survival is unlikely.

If the dose of irradiation is not excessive, vascular degeneration is apparently followed by a phase in which a more or less normal circulation is re-established despite the appearance of telangiectasia. The rapidity and extent of recovery may depend on the severity of the initial trauma. This recovery is indicated by a return toward normal of the time required for autografts to revascularize and by a sharp rise in the percentage of survivals.

SUMMARY

An autogenous skin transplant upon a rabbit's ear may be shown to require between 4 and 5 days for effective revascularization. Roentgen irradiation in doses sufficient to produce severe erythema is apparently capable of influencing both the revascularization time and the ultimate survival of such grafts.

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VASCULAR REINFORCEMENT OF PEDICLED TISSUES QUANTITATION BY ARTERIOGRAPHY OF INCREASE IN *CIRCULATION OBTAINED USING HISTAMINE IONTOPHORESIS**

CLAYTON R DEHAAN AND RICHARD BOIES STARK

In a previous publication, one of us[1 2] presented preliminary data which suggested that the vascularity of pedicled tissues could be increased by artificial means Many agents and modalities were used Inflammation induced with live contaminants and sterilely by vaccines, priscoline, ultra violet, intermittent mechanical constriction of the pedicle, surgical tran section, and histamine induced subcutaneously by injection and percuta neously by iontophoresis The degree of vascular augmentation achieved was assayed (1) by the amplitude of the pulse waves of a pedicle that is sealed hermetically within the closed chamber of a digital plethysmograph (an instrument which measures changes in the volume of the pedicle per unit of time), and (2) by transilluminating a rabbit ear pedicle (auricular circle) and recording vascular changes by photography Of the methods tried, that which offered the greatest promise of increasing circulation of pedicled tissues was histamine induced by iontophoresis

This study compares the vascular changes in pedicled tissues that occur as the result of aging unmolested with vascular changes that occur in the wake of hyperemia induced by histamine iontophoresis Although the earlier experiments were performed without prior knowledge, the author

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Aided by grants from the U S P H S (H 2642) the W Alton Jones Foundation and the John A Hartford Foundation Inc
With the technical assistance of Mrs Joan Taylor and Mr W Harry Brownlee

acknowledges belatedly the previous use of histamine by Prudente,[3] who used this agent as a skin inunction prior to transplanting pedicled flaps

METHOD

1. Formation of Pedicles. Bilateral tubed pedicles were formed longitudinally across the ventral flexion crease of the groin of Flemish giant rabbits Pedicles were placed in this region because the area lends itself readily to angiographic studies if the aorta is cannulated Because of the limited surface area in the groins, tubed pedicles were somewhat smaller (3x1 5 inches) than those used in our preliminary report (5x2 5 inches), but the length to width ratio remained the same

2. Histamine Iontophoresis. Histamine was induced into the dermis and subcutaneous tissue by ion transfer The skin of the tubed pedicle was clipped free of hair and cleansed with ether Histamine dihydrochloride (1%) in an ointment base was applied to the skin of the tubed pedicle The positive pole of the galvanometer was placed over the tubed pedicle The metal electrode was kept from direct skin contact by a moist felt pad which in turn was wrapped with a moist paper towel so the ointment would not soil the felt An indifferent or dispersing electrode with felt pad soaked in saline was placed on the clipped skin of the nuchal region The current was increased slowly up to 5 milliamperes and left uninterrupted for 10 minutes Thus, by uni directional flow of galvanic current the histamine was driven out of solution and into the skin, via the ductal orifices where it produces a powerful vasodilatory effect

3. Arteriography. Our initial attempts to visualize the arterial tree of isolated pedicled segments in the live animal were unsuccessful We had hoped to obtain unequivocal serial arteriograms within the same animal which would allow us to follow vascular changes wrought by time and treatment Conventional radiopaque agents (50% sodium hypaque and 70% sodium urokon) produced little if any visible filling and, hence, were abandoned Thorium dioxide (Thoratrast) gave results which were somewhat better, but a relatively small amount of this radioactive solution (5 cc) proved lethal to the animals

As a consequence the animals were sacrificed and the distal abdominal aortas cannulated Dispersing and retrograde flow of dye were prevented somewhat by ligating the inferior vena cava and the femoral vessels in the thigh All injection agents used produced poor arteriograms with the exception of thorium doxide, but this agent produced usable arteriograms only if 30 to 40 cc were used

4. Vinyl Plastic Casts of the Vascular Trees. Three dimensional casts of the vascular trees of tubed pedicles were made Vinyl plastic (vinyl acetate) was injected into the large arterioles of the lateral abdominal wall After the plastic material had become firm, the pedicles were resected and placed in a digestion bath of 10% sodium hydroxide Within 48 to 72 hours the soft tissues were digested, leaving behind a positive mold of the vascular tree

Effects of Histamine Iontophoresis on the Blood Vessels of Tubed Pedicles.
1 Results of arteriography Histamine was administered to the skin of tubed pedicles Almost without exception, treatments were given upon



Fig 2 Three dimensional vinyl acetate casts of the vascular trees of tubed pedicles of the same age (12 days) formed bilaterally in the groin of a rabbit The soft tissues of the tubed pedicles have been digested away leaving behind the positive impression of the vascular trees The tubed pedicle on the right (12 d) is the control whereas that on the left (8Rx) has been treated once daily for 8 days with histamine iontophoresis

The cast of the vessels of the control tubed pedicle resemble a grape twig while that of the vessel of the treated tubed pedicle resemble a veritable sea sponge

vessels (particularly in the smaller vessels of the dermis of arteriolar and capillary size) as well as the main cutaneous vessels In graphic language, the difference between the untreated vascular trees and those treated with histamine was as great as comparing a grape twig with a sea sponge

DISCUSSION

The vascular supply to skin is composed of branches of vessels which supply muscles These branches penetrate the subcutaneous tissue as the main cutaneous arteries and veins, and these enter the deep dermis (reticular layer) Here they anastomose with one another, forming the cutaneous network[4] These vessels traverse the reticular layer of the dermis In the papillary layer of the dermis they run parallel to the skin surface and give off multiple, small vascular tufts to the dermal papillae These connected tufts form the subpapillary plexus, which has been demonstrated beautifully by Braithwaite[5] The morphology of the cutaneous arterial network might be likened to a candelabra which gives off from the reticular stem 10 to 12 vascular tufts to the rete pegs of the dermis This vascular branching takes place in 3 dimensions It is these far flung vascular connections of the subpapillary plexus which account for the ability of the skin to survive subtotal avulsions and wide surgical undermining

The vascular changes that manifest themselves upon arteriography following formation of a tubed pedicle which was not treated are most marked during the second week of its existence They consist first of vascular hypertrophy, but by the tenth day, an increase in vessel length is manifest This lengthening of vessels offsets twisting and stretching of vessels that may accompany transplantation leading to ischemia The vascularity of tubed pedicles appears to diminish slightly after the second week of its existence

Histamine administered by iontophoresis enhances the vascularity of tubed pedicles in several ways It produces a startling hypertrophy of existing vessels Also it produces a marked hyperplasia of the subpapillary dermal plexuses which are not otherwise visible upon arteriography In

This paper is a report of one part of an extensive experiment designed for the study of the histologic and histochemical changes which occur in burned skin and in healing burns. It deals particularly with alterations of the dermal collagen *after* new epithelium has covered the surface of a moderate second degree burn.

METHOD

Young Chester White pigs were selected as the experimental animals because of the histologic similarity between their skin and human skin. They were anesthetized with intraperitoneal Dial in Urea urethane (Ciba) in doses of 60 to 70 mg/kg of body weight. Their hair was closely clipped and the skin was gently washed with water and a liquid detergent.

The heat source for the small area burns was a modified 24 inch Army carbon arc searchlight,[1] and a burning magnesium source was used to produce large area burns.[3] With the carbon arc, 576 radiant exposures of 10 calories per square centimeter were made. Some of these were produced with a rectangular thermal pulse and others with a pulse simulating that of the atomic bomb. Exposure times varied from 0.3 sec. to 12.6 sec. The diameter of these burns was 1.7 cm. They were biopsied at one of the following times after injury: 4 hours, 1 day, 2 days, 4 days, 5 days, 9 days, 10 days, and 14 days. A method of random assignment was determined when a given burn would be biopsied; there was, therefore, equal probability that any one burn of a particular type would be biopsied at any of the above times.

With the magnesium source, 36 uniform, moderate second degree burns with a diameter of 3 inches were produced on 9 animals. Each of 12 of these large area burns was biopsied at the following times after injury: immediately, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 16 hours, and 48 hours. Each of another 12 burns was biopsied at 1, 3, 6, 8, 10, 13, 15, and 17 days after burning. Each of the remaining 12 burns was biopsied at 1 hour, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 6 weeks after injury. Again a random assignment assured equal probability of any one area of a given burn being biopsied at any of the selected biopsy times.

All specimens were stained by a method for the differential staining of burned and normal tissue.[2] Additional sections of most of the burns were prepared by stains for mucopolysaccharide, for reticulin and for collagen.

RESULTS

Only a summary of one of the most interesting facets of this study is presented here.

Soon after the production of a moderate second degree radiant energy burn the involved area is infiltrated by leucocytes. Between 24 and 72 hours after injury the damaged tissue is effectively isolated from the unharmed dermal tissues. As epithelium regenerates from the periphery and from the hair follicles the new epidermis covers dermal fibers which have the morphologic appearance of, and take the stains for, normal dermal collagen.

Shortly after epithelial coverage, however, the dermis undergoes profound changes in the following sequence: capillary proliferation and leuco-

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Soon after the production of a moderate second degree radiant energy burn the involved area is infiltrated by leucocytes Between 24 and 72 hours after injury the damaged tissue is effectively isolated from the unharmed dermal tissues As epithelium regenerates from the periphery and from the hair follicles the new epidermis covers dermal fibers which have the morphologic appearance of, and take the stains for, normal dermal collagen

Shortly after epithelial coverage, however, the dermis undergoes profound changes in the following sequence capillary proliferation and leuco-

process is already present and will be most prominent during the next week or two Between 2 and 5 weeks after injury the maximum staining of new fibers by reticulin stains occurs and from 3 to 6 weeks the new fibers assume the characteristics of collagen In mild second degree burns the restoration process begins early and is nearly complete at 6 weeks but in more severe burns the superficial dermis that which first undergoes a change is still being repaired (Fig 2)

DISCUSSION

The alterations described above accomplish at least two things First the proliferation of tissue in the unburned areas acts simultaneously with the fibrous proliferation in the subcutaneous tissue to restore the dermis to normal thickness Indeed the early result is often a dermis somewhat thicker than normal The second accomplishment is the early formation of a loose dermal layer into which the rete pegs of the epidermis are able to grow with ease and the later development of a superficial dermal layer resembling that of the original stratum papillare

There seems to be striking mutual influences between new epidermis and dermal collagen Epithelial coverage is followed by a rebuilding of the dermis During the breakdown of dermal collagen an abnormally thick epithelium with long blunt rete pegs develops As the dermis is restored toward normal the epidermis assumes a more nearly normal appearance A failure of these processes may be responsible for the smooth epithelial coverage of dense collagen bundles seen in keloids and hyper trophic scars

SUMMARY AND CONCLUSIONS

Although a burned area is often considered healed when epithelial coverage is complete it is a common clinical observation that such an area changes its appearance during the subsequent weeks and months Some histologic and histochemical changes in the epithelium covered burned area are described The apparent influence of epidermis and dermal collagen on each other during the periods of breakdown and restoration is pointed out This rebuilding of normal body tissues would indicate a continuing need for nutritional supplements long after the accomplishment of epithelial coverage of large area burns A failure in the reparative processes described may be responsible for the formation of hypertrophic scars and keloids

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THE MARGINAL LOCALIZATION OF THE CONTRACTION MECHANISM IN OPEN WOUNDS*

HERMES C. GRILLO, GEORGE T. WATTS AND JEROME GROSS

Full thickness skin defects in mammals heal in part by contraction. The completeness of contraction depends upon many factors such as wound location and size. The forces responsible for contraction of open wounds have generally been held to originate in the mass of granulation tissue which forms in the defect. These have variously ascribed to tensile forces arising from collagen fiber shortening, diminution in wound contents, or to an undefined pull of the granulating mass.[1,3,5,8] This study was undertaken to define the role of the granulation tissue in the contraction mechanism.

Full thickness wounds measuring 2.0 cm. square through the skin and panniculus carnosus were made on the backs of guinea pigs. The corners and midpoints of each side were marked by tattoo points.[1] This is necessary in order to outline the wounds accurately since the midpoints of the sides move more rapidly than the corners and since epithelialization proceeds more rapidly than wound edge movement. The areas were traced directly and measured by planimetry. The wound contents were excised along lines defined by the tattoo points and from the base of deep fascia. Wounds were analyzed periodically up to 30 days for total weight of tissue, water, hydroxyproline,[9] hexosamine,[4,7] and tyrosine.[2] Hydroxyproline was used as an index of collagen content, hexosamine as an index of mucopolysaccharides and glycoproteins, and tyrosine as an index of noncollagenous proteins.

Contraction in wound area begins at once but does not become uniform until the third to the fifth day. The wound is 70% contracted by the tenth day and contraction is essentially complete and results in full wound closure by the fifteenth day apart from the epithelialization process (Fig. 1).

When the total amount of wet tissue present in the wound is measured a rapid increase in total new formed tissue is noted from the third to the eighth day. The amount of wet tissue then declines sharply, the curve flattening out at 3 weeks. Comparison of the time curve of contraction with that of total wound content does not reveal a parallel relationship. In fact, both rapid increase and decrease in wound content occurs during a period of continuous contraction.

The *total water content* varies with time in the same way as total wound contents. The *concentration of water* in the whole tissue shows only a 1% linear fall in 30 days.

The *total amount of collagen* begins to rise sharply at 5 days to a maximum at 8 days, then there is a fall in amount, initially rapid up to 10 days and then more slowly. A lack of correlation between the contraction process and the total amount of collagen in the wound is evident

*From the Robert W. Lovett Memorial Laboratories and the Department of Surgery of the Massachusetts General Hospital and the Harvard Medical School. Supported in part by research grant C 3638 from the National Cancer Institute and research grant A90 (C7) from the National Institute of Arthritis and Metabolic Diseases of the United States Public Health Service.

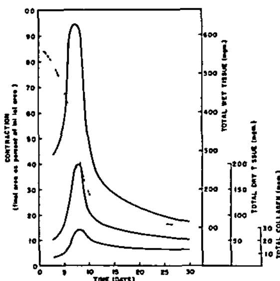


Fig 1 The dotted line indicates the progress of contraction Of the 3 unbroken curves the top indicates total wet tissue in the wound, the middle line total solid tissue in the wound and the bottom total collagen in the wound The curves are plotted from mean data of 3 to 8 wounds on days 3 4 5 7, 8 11, 14, 17 20 25 and 30 Different scales are used for convenience The data has been corrected for deviation in initial wound sizes It is to be noted that the percentage of solid material remains nearly constant

Although the proportion of solids in the wound tissue remains essentially constant, the distribution of components varies The *concentration of collagen* rises rapidly from the fifth day to about the end of the second week after which it approaches the concentration found in normal skin

Hexosamine concentration falls continuously in the first 10 days from values close to serum levels at 24 hours toward levels found in normal skin Tyrosine levels fall from the third day toward normal skin levels

These data show that contraction follows a course independent of the total amount of tissue filling the defect at various stages The relatively unchanged water concentration of the wound makes it apparent that loss of water is not the cause of the contraction process The early peak in total amount of collagen followed by rapid decrease, lacking direct correlation with the curve of contraction, suggests that the two are unrelated The curve of increasing collagen concentration alone shows possible partial coincidence with the contraction curve

The continuous fall in hexosamine suggests that the hexosamine enters the wound primarily in the exudate and is in large part reduced as the exudate is removed This might be expected since hexosamine is present in relatively large amounts in serum as a component of glycoproteins as well as being an index of mucopolysaccharides Tyrosine values would seem to support this contention

Since no relationship to explain the process could be found between contraction and total tissue, water, collagen, hexosamine and tyrosine content and concentration, we were reluctant to accept the view that the granulation tissue filling the wound acted as a contracting unit Our doubts were reinforced by the observation of a 'picture frame' of dense white connective tissue beneath the wound edge and strongly adherent to the fascial base, which progressed with the skin edge as contraction proceeded This focused attention on marginal activity

Using the same type of wound as described earlier, a series of morphologic experiments was performed to clarify the role of granulation tissue in contraction and to localize the site where the force for contraction lies Two wounds were made in each animal and one used as a control Different

regions and amounts of the wounds were excised usually at the seventh day when the granulation tissue was well established and when active uniform contraction was going on

Excision of Central Granulations A narrow fringe of granulations was left around the margin of the wound The granulation tissue within this line was cleared completely down to the original base of the wound In other animals the excision was repeated successively whenever any granulations were visible The rate of contraction was not influenced by these excisions It was also noted that the wound size did not increase after the excision

Excision of Entire Granulating Area Excision was carried up to the edge of the skin margin and down to the original base There was difficulty in excising the granulations without detaching the skin from the base outside the line of the incision The results are not as clearcut as in the previous experiment In some cases contraction was delayed but in others no change occurred and the wound continued to contract as though no intervention had taken place It was not possible to decide whether the delay was caused by a detachment of the wound margin from the base or whether the zone of activity actually extended in some cases into the outermost granulations so that it was damaged by the excision Again the wound did not enlarge at the time of the excision of the granulations

Excision of Granulations and Skin Margin Excision included 0 5 mm of the original skin margin and all the granulations This caused immediate distraction of the wound area to a size greater than that of the original wound (not the contracted wound) by about the area of additional skin removed The tissue encompassed by the incision did not get smaller in area when removed Following the incision contraction began anew along a curve similar in slope to the original It is unlikely that the elements causing contraction are acting outside the marginal skin

Excision of Wound Margin Only The excision removed a narrow strip *of wound margin including skin edge and adjacent granulations* but left the central granulations still *in situ* The results were the same as in the previous experiment

DISCUSSION AND SUMMARY

These results indicate that the mechanism of contraction in full thickness skin defects does not lie in the granulation tissue filling the center of the wound Neither are the edges pushed in by a process occurring in the peripheral tissues The machinery of contraction must therefore lie in a narrow zone underlying the advancing skin margin — the picture frame This thin strip of new formed connective tissue binds the wound edge firmly to the fascia of the wound base Histologically it appears to be a very cellular mass containing a little collagen but consisting mainly of fibroblasts

This work does not deny the role of collagen in wound healing either in relation to the development of tensile strength[6] or in relation to late cicatrization of wounds Nor does it negate the possible role of newly *forming connective tissue at the wound margin in contraction* But both the chemical and morphologic studies indicate that the central granulation

tissue does not act as a unit to cause contraction and that it is also quite unnecessary for the contraction process The mechanism seems to be localized in the margin of the wound One might postulate mass cell movements pulling the free skin edge However, precisely how the con traction force originates is still an open question

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STUDIES ON ACQUIRED TOLERANCE TO HOMOGRAFTS AND HETEROGRAFTS*

RICHARD H EGDAHL FRANKLIN D ROLLER AND RICHARD L VARCO

One of the most exciting recent developments in transplantation research has been the discovery of the principle of acquired tolerance by Billing ham Brent and Medawar in 1953[1] These workers have convincingly dem onstrated in mice that injection of the fetuses or newborn animals of one strain with cells from adult members of another strain of mice will so alter the injected animals that at maturity they will accept permanently skin homografts from the donor strain This acquired tolerance to the cells (or antigens) which were injected early in the development of these mice is specific they retain a normal immunological reactivity to other antigens This principle of acquired tolerance has subsequently been confirmed for simple protein antigens bacterial products[3] and tumors[4 5] Woodruff[6] has achieved acquired tolerance to skin homografts in rats by

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neonatal cell injections Thus far, his work has not been confirmed One purpose of this study has been to produce acquired tolerance to skin homo grafts in the rat colony used in our laboratories A second part of the study was directed toward the problem of heterografting, and the possibility of achieving *heterologous tolerance* Previous work in this subject has been limited to various fowl combinations [7, 8] Here it is difficult to define the degree of heterogeneity We have used rats and rabbits in experiments on heterologous tolerance, feeling that these dissimilar mammals represent a thorough test of the establishment of "acquired tolerance" in a hetero graft system

METHOD

Both Sprague Dawley rats (from Holtzman, Madison, Wisconsin) and hooded rats (from Pacific Coast Laboratories) were used throughout in these studies Effort was made to obtain pregnant rats in the second or third pregnancies for the *in utero* experiments A much lower incidence of abortion was found in these animals, compared to those in a first pregnancy New Zealand white rabbits, weighing 2 to 6 kg, were selected for the dermal heterograft A full thickness skin graft 2 cm square was removed from the rabbit host and placed on the abdomen of the rat This was dressed with a gauze sponge and then covered with a few turns of 2 inch tape It was changed and the grafts visually examined every 2 days

The cell suspensions were prepared from the spleen of either rat or rabbit The organ was placed in limited volume Ringer's solution to which a drop of heparin had been added, and the contents gently teased free with a hypodermic needle The resultant thick suspension of cells and tissue debris was filtered through gauze, and the final cell suspension would easily pass through a #30 needle Viability of cells was determined by studying the uptake of Trypan blue, and cell counts were performed in the usual manner

In the homografting experiments, some newborn rats were injected intraperitoneally or intravenously with 0 1 to 0 25 cc of the spleen cell suspension from other adult rats, either of the same or of different strain Alternatively a tissue mash of spleen from a comparable source was injected intraperitoneally with a trocar *In utero* injection of rabbit spleen cells was performed in rat fetuses from 1 to 5 days before term A lower midline laparotomy was used in order to expose both uterine horns An injection of 01 and 025 cc was made, usually into the peritoneal cavity, but in a few instances the injection was into the sagittal sinus The latter seemed to induce a higher abortion rate, and certainty about these injections was less In some heterografting experiments additional "booster" doses of heterologous cells from lymph nodes of the same donor rabbit were given intraperitoneally after birth to animals previously injected *in utero* After animals prepared by an early exposure to donor antigen in both homo grafting and heterografting experiments were at least 6 weeks old they were grafted with skin from the original donor The results of these studies have been compared to a similar series of homografts and hetero grafts performed in a control series of uninjected animals

RESULTS

The results of experiments on homologous tolerance are summarized in Table 1 The control rat homografts survived on the average 11 2 days,

Table 1 Acquired Tolerance in Rats Induced by Newborn Injection of Homologous Spleen Cells

NO OF RATS	TIME OF PREPARATION INJECTION	NUMBER OF VIABLE CELLS INJECTED	% TOLERANCE
19	Cell suspension	127 225 million	58
24	Spleen mash	Not counted	83

and the longest 'take' was for 2 weeks On the contrary, in this present study the animals were considered tolerant when comparable homografts remained viable and unaltered for longer than 2 months Justification for this assumption is derived from the continued persistance of such tolerant homografts long past this interval The rats were grafted at 2 months of age, since it has been shown that at this age this animal rejects homografts at a relatively constant rate

Our data demonstrate that the intraperitoneal injection of a spleen mash" is more effective than cell suspensions from the same source in producing homograft tolerance Although cell counts of this brei were not secured, obviously several times the concentration of cells existed here as compared to cell suspensions described earlier Interestingly, about 75% of the newborn rats that had been injected with pulpy mixture died within the next 5 to 10 days Delayed deaths after injection of the cell suspension were only infrequently observed

Table 2 summarizes the results of experiments on heterograft tolerance In a control series of rabbit to rat heterografts, the mean survival time was 5 9 days, and the longest was for 7 0 days In the experimental group partial heterologous tolerance was achieved, therefore, several heterografts survived well beyond the control period

Table 2 Partial Acquired Tolerance in Rats Induced by Injection of Heterologous Spleen and Lymph Node Cells

NO OF RATS	DAYS BEFORE DELIVERY (INJECTION)	SURVIVAL OF HETEROGRAFT (DAYS)	COMMENT
1	2	23	1 of 30 animals in 6 litters
2	4	17 and 13	2 of 16 animals in 4 litters
2	2	11 and 14	2 of 14 animals in 4 litters
5	2	13	Injected with lymph node on first and third days after birth in addition

DISCUSSION

These studies fully confirm Woodruff's findings that homologous acquired tolerance can be produced in rats by the intraperitoneal injection into newborn rats of homologous cells The large mortality at 4 to 10 days in newborn rats injected with cellular tissue mixture is reminiscent of Billingham, Brent, and Medawar's findings in experiments on acquired tolerance in newborn mice[9] It is possible that the homotransplanted splenic cells survive in a sufficient number to produce antibodies against the newborn host and "overwhelm" it in some manner We do not have studies of the reticulo endothelial system of these injected rats which would clarify this point

Although a comparable degree of heterologous acquired tolerance was not achieved, several rats exhibited prolonged heterograft survival The failure to induce long lasting heterologous tolerance is most likely due to our inability to introduce sufficient donor specific antigen at an early enough stage in the animals' immunological development However, the concept of "the earlier the better is not necessarily the entire story[10] Al though we have demonstrated that the intraperitoneal injection of massive numbers of heterologous cells will not result in partial tolerance in new born rats, we have found that repeating the injection of donor spleen cells after birth appears to increase the percent of animals developing partial heterologous tolerance, but not the length of heterograft survival These studies are continuing, and results of retransplantation and larger *in utero* cell inoculations will be reported at a later date

CONCLUSIONS

1 Acquired tolerance to homologous skin has been produced in rats by the intraperitoneal injection of spleen cells into newborn animals

2 Prolonged survival of skin heterografts was achieved in rats by the *in utero* injection of rat fetuses with rabbit spleen cells

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STUDIES ON REVERSIBILITY OF HOMOGRAFT REJECTION*

MILTON T EDGERTON AND PATRICIA J EDGERTON

One of the major theories for the homograft rejection phenomenon is that it is *basically an antigen antibody reaction It is well known that skin* homografts will immunize or sensitize a host to later grafts, if left on until the graft is rejected It has not been clear, however, at what time the skin graft itself *first* becomes irreversibly affected by host resistance A second question raised is whether the host will be fully immunized if a homograft is removed *before* the rather sharp end point of graft rejection

To determine these points, the transparent chamber technique has been utilized to study the reversibility of homografting A line and CBA inbred mouse strains were selected for interstrain homografting A line grafts were placed as homografts on CBA mice and left on for varying periods, ranging from 1 to 9 days A careful control series of over 300 homografts between these strains revealed a classic end point of nine days (± 0 2 days) as judged by transparent chamber criteria †

The skin grafts were observed microscopically each day for graft vessel pattern, circulation, hair growth, and gross changes They were then gently peeled and returned to the original strain as isografts in order to confirm their viability and potentiality for complete recovery

The results were clear cut and somewhat surprising They are shown in Table 1

Table 1 A jax Skin Grafts on CBA Hosts, Returned After 1 to 9 Days to A Jax Host to Test Viability

A-GRAFT → CBA HOST		THEN →	CONDITIONED A GRAFT → A HOST
NO OF DAYS AS HOMOGRAFT	NO OF MICE IN EACH GROUP	NO OF GRAFTS SHOWING CIRCULATION AS ISOGRAFT	NO OF GRAFTS SHOWING PERMANENT SURVIVAL AS ISOGRAFT
1	2	2 (100%)	2 (100%)
3	3	3 (100%)	3 (100%)
5	6	6 (100%)	6 (100%)
6	6	6 (100%)	6 (100%)
7	9	7 (78%)	6 (66%)
8	5	5 (100%)	None (0%)
9	11	*None (0%)*	*None (0%)*

†Sudden slowing of flow in graft vessels and reduction in calibre of larger vessels (is usually followed by gross signs of graft failure within twenty four hours)

*From the Division of Plastic Surgery The Johns Hopkins Hospital and The Johns Hopkins University School of Medicine Baltimore Supported in part by U S P H S Grant RG 3960 (C3) the W Alton Jones Foundation and private contributions to Consolidated Gifts in Plastic Surgery

In general skin grafts could "take" and live up to 6 days as homografts and still be returned to parent strain as an isograft with 100% survivals If left on 7 days the grafts would recover and survive approximately two-thirds of the time on re-isografting If left on 8 days the grafts would develop circulation on return to original donor strain but *none* of the grafts survived permanently Grafts that were left on as homografts until the 9th day never regained circulation with re-isografting and none survived

Conclusions This data showed rather clearly that certain changes occurred to the homografts 24 to 48 hours *before* the sharp classic end point These changes were sufficient to interfere with the viability of the grafts with great consistency – even though they were returned to isogenous histocompatible environments The inference is strong that some substance or substances entered the grafts and brought about a cellular death or weakening on the seventh to ninth days of homografting

A second pertinent question raised is whether the hosts were immunized or affected in a significant way by the short term contacts with these homografts or is the gross death of cells in the graft (at or after rejection end point) necessary to set up these immune mechanisms?

Our evidence is clear that the hosts are definitely immunized by short term contacts Certainly hosts that bear homografts for 7 days are completely sensitized even though the grafts are then removed while looking perfectly healthy, both microscopically and grossly At present our data would suggest that even hosts bearing grafts for as short a period as 4 days are also "immunized" but further studies are now in progress to establish the minimum period for this effect

Conclusion Once again these results suggest that some product from the homograft enters the host at a very early time (certainly well before the gross rejection point) and in sufficient quantity to produce the condition of readiness or resistance in the host that is in every way like that of classical immunization Second set homograft rejections occur in these animals even though the initial homografts are never allowed to slough

The question was then raised as to whether these short term exposures of homograft to host would result in gradual devitalization of the graft or if by rapid serial transfers the homografts could be kept alive indefinitely and perhaps even "conditioned" to permanent tolerance of the new host tissues

Again CBA and A line mice were used in a series of experiments Homografts were placed from A line mice on CBA hosts and left in place from 1 to 7 days These grafts were then transferred to second and later third and fourth hosts – moving them each time by a careful "peeling" and observing each step of the way by transparent chamber techniques Unless clear circulation within the vessels of the graft itself was seen the graft was not considered a "take"

Five series of mice were each used as host in series for a single homograft The graft was left on each host for 4 days and then transferred to the next In each instance the "open technique" and transparent chamber methods[3] were used to study the grafts

After four regraftings of each homograft it was left in place on the final host and observations made of the rejection time and intensity

A striking prolongation of homograft survival occurred The average healthy life of these homografts was 23 0 days (± 0 5 days) as compared with the control group in which the grafts were regularly rejected on the ninth day (± 0 2 days) This represented an increase in healthy graft life of 150% In addition the homograft rejection point was delayed from 9 to 11 days (22%) on the final host alone

In attempting to explain these increases we wondered if the rapid transfers prevented circulatory communication between graft and host, and in this way slowed the homograft reaction However, the transparent chamber studies showed that each graft had become clearly vascularized *on each host* and that circulation was clearly seen in the graft vessels The total number of days of this circulation was 16 0 days (± 0 5 days) as compared with 7 days of circulation in the control or nontransferred grafts

A surprising feature of the results was the consistent finding that circulation and vascularization within these skin homografts proceeded more rapidly with each transfer to a new host It was as though the previous grafting had placed the vascular bed of the graft in a state of readiness Often on the fourth grafting the circulation in the graft was clearly seen on the day after grafting

DISCUSSION

In discussing this problem with Dr E J Eichwald, the questions were raised Can a homograft be kept alive indefinitely by regrafting onto new host before the rejection mechanism has an opportunity to damage the graft? Will the short periods of circulation on each host be sufficient to furnish the necessary oxygen and nutrients to maintain a state of health in spite of the insults of surgery? And, finally, will these interrupted periods of contact between graft and host permit the gradual conditioning of the graft to the host tissues (possibly by a change in the protein structure of the graft)? If conditioning is possible, may it be possible to leave the homograft on a final host and have it survive permanently?

More work is needed to answer the above questions categorically, but, at present, we have not yet found any limit to the length of time that a homograft may be kept alive and healthy, if it is transferred at sufficiently short intervals from one non immunized host to another To date, we have had an increase in homograft rejection from 9 to 31 days by carrying the graft on 4 hosts With gentleness and aseptic precautions it is certainly possible to carry out multiple regraftings without infection

SUMMARY

It is clear that multiple transfers of skin homografts at 4 day intervals will increase the tolerance between graft and host This probably results from a progressive decrease in the antigenicity of the grafts This increase in tolerance is *not* due to any lack of intimate circulatory contact between host and graft that might result from the trauma of surgical transfer

The modern concepts of high speed protein shifts suggest that the short periods of graft host contact permit dispersal of graft antigen into the host and thus reduces the individuality of the graft It is also possible that host proteins enter the graft with each new host and bring about a gradual replacement of the original graft proteins

At present protein tracer studies are under way to help answer some of the questions

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PULSATILE ACTIVITY IN TOTAL TRANSPLANTATION OF EMBRYONIC MOUSE HEARTS AS AN INDEX OF SURVIVAL OF HOMOTRANSPLANTS*

HERBERT CONWAY B HEROLD GRIFFITH JOHN E SHANNON AND ANGELICA FINDLEY

In order to have a means of determining the length of time that individual cells of a homograft may remain viable we have used the embryonic heart as our experimental tissue The pulsations of the homografted heart as observed in the transparent chamber provide a ready means of identification of the transplanted cells and also serve as an unquestionable indicator of their viability The cessation of the pulsations is a clearcut end point of the survival time of the graft

In this paper the technique of transplanting embryonic hearts and the methods employed in their study are described Observations based on the study of transplanted hearts and data on the survival time of homografted cells are presented Some preliminary data on the experimental treatment of homografted hearts are also included

METHOD

The Saran window modification[1 2] of the transparent chamber technique was employed The beds which were to receive the grafted embryonic hearts were prepared in the skin of the mid dorsum of the mouse exactly as described for grafts of full thickness skin Each intact embryonic heart was placed directly over a large host vessel lying in the carefully exposed panniculus carnosus The cellulose acetate ring with a clear thin film of Saran plastic was then affixed to form the transparent chamber In a few cases instead of the plastic window a natural window was prepared by applying an autograft of thin auricular skin to the open bed About three weeks later the embryonic heart was then carefully inserted between the panniculus adiposus of the established autograft of ear skin and the panniculus carnosus of the dorsal skin fold

The hosts were mice of strains A C57Br C57Bl C3Heb and Swiss albino and ranged in age from three months to one year

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The transplants were the intact hearts of embryos obtained from mice on the 10th day to 18th day of gestation In some cases, intact hearts removed from newborn mice 1 to 3 days of age were used The age of the embryos was estimated from the stages described by Gruneberg[3] The majority of the embryos that were used were from 11 to 17 days of age

To obtain the embryos, a pregnant mouse was sacrificed by cervical dislocation and the uterus was excised aseptically. The embryos were removed from the uterus, freed of all membranes, and placed on the stage of a dissecting dish Each heart was dissected out rapidly, placed in a balanced salt solution, and then inserted into the transparent chamber A balanced salt solution such as Earl's or Gey's was found to keep the hearts in better condition than did either Ringer's solution or isotonic saline

Almost half of the hearts received no special treatment before or after transplantation. However, studies were made on some hearts which were subjected to freeze thawing before transplantation and on others which were perfused with various solutions after transplantation

For freeze thawing, the following technique was used Immediately after excision, the heart was placed in a test tube containing 0 2 ml of a solution of 25% glycerol in Eagle's Medium #199, which had been prewarmed to 38°C It was then incubated at 38°C for 15 minutes The use here of the glycerol solution to protect the cells as much as possible from the damage incurred during freezing was adapted from the work of Smith[4] According to the findings of Lovelock[5] the glycerol in the cells acts as a "salt buffer"

After incubation the heart was quickly frozen by plunging the test tube into a bath of alcohol and dry ice at 78°C The temperature was carefully maintained and the heart was frozen for 30 minutes The heart was then thawed rapidly by immersing the tube in a water bath at 38°C, and simultaneously rinsing the heart with the mixture of glycerol and Medium #199, which was also at 38°C Immediately after thawing, the heart was inserted into the transparent chamber

The perfusion technique was as follows the heart was placed in the transparent chamber immediately after removal from the embryo Twenty four hours after transplantation, 0 1 ml of an experimental solution was instilled into the chamber once a day for 5 days The experimental solutions which were used were Earle's Balanced Salt Solution, 02 M L glutamine, and a 10X dilution of Eagle's Vitamin Concentrate Stock B for synthetic media

In all cases, the hearts were observed at least once a day with transmitted light under a stereoscopic microscope

RESULTS

Eighty nine homotransplanted hearts were studied Of these 52 were given no special treatment, 18 were perfused after transplantation and 19 were subjected to rapid freezing and thawing before transplantation We shall describe the phenomena which were noted in the course of observing the hearts

By the fourth postoperative day when vascular connections with the hosts were established all the hearts had resumed beating In general, the rate of pulsation of the transplanted hearts remained remarkably

constant from almost the fifth day to about the sixteenth day Rates of 200 to 300 beats per minute were not uncommon The normal heart rate of adult mice is 400 to 600 beats per minute After the sixteenth day the rate of pulsation gradually diminished and stopped abruptly at about 18 days in most cases By approximately the fourteenth day after trans plantation the outline of the hearts had become very faint and it was obvious that the transplants were undergoing absorption Nevertheless vigorous pulsations of the transplanted tissue persisted in most cases In some hearts, however, only one auricle was beating regularly at this time and the ventricles were contracting intermittently These findings were interpreted as indicating that, even though a portion of the transplant was evidently necrotic and was undergoing dissolution, there were areas of the grafted tissue which remained viable for a longer period Histologic studies substantiated this interpretation

Sections of transplants made on the fourteenth postoperative day when pulsations were still vigorous showed a pronounced round cell infiltration of the heart Although the morphologic integrity of the heart was largely obliterated, there remained some semblance of tissue organization and a few areas of the myocardium had a completely normal appearance

Sections of transplants made as soon as all pulsations ceased showed more diffuse round cell infiltration and all of the myocardial cells appeared to be necrotic These findings suggest that as long as any cells remain viable they will contract It is obvious that there is a great advantage in using the heart as the transplant in studies on homograft survival since the viability of even very small areas can be detected

Twenty four hearts were fixed for histologic studies at various intervals The 28 remaining hearts were allowed to continue until all pulsations ceased The median survival time of the untreated embryonic heart homo grafts was calculated to be 19 3 days

The 18 hearts which were perfused after transplantation and the 19 hearts which were frozen and thawed before transplantation behaved similarly with a few exceptions The median survival time of the perfused hearts was 12 5 days and that of the freeze thawed hearts 13 0 days One homograft treated by freeze thawing continued to pulsate rhythmically for 35 days One untreated graft retained its morphologic integrity and con tinued to pulsate vigorously for 15 days

At no time during our studies of the heart transplants did we observe the regular sequence of events previously described as a good criterion for the survival time of skin homografts[1 2] namely dilation of blood vessels in the graft slowing of blood flow, and the formation of thrombi The median survival time of skin homografts as determined by these criteria was found to be 9 5 days

SUMMARY

In summary this is the presentation of work in which embryonic hearts of mice have been homotransplanted to the dorsal skin fold of unrelated recipients This particular tissue has been found to be ideal for the inter pretation of the survival time of homotransplanted tissue because the ces sation of cardiac pulsations affords a very definite end point Also the pulsatile activity of a transplanted embryonic heart definitely identifies the

transplanted tissue from the infiltrating tissue of the recipient Untreated embryonic hearts exhibited a median survival time of 19 3 days Embryonic hearts which had been perfused showed a median survival time of 12 5 days Embryonic hearts which had been frozen and thawed before trans plantation had a median survival time of 13 0 days The homotransplanta tion of embryonic hearts using a tissue chamber technique is presented here as an ideal method for the assay of the exact effect of experimental pretreatment of homografts on their survival time Other studies are in preparation

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TISSUE REACTIONS TO CROSS GRAFTS FOLLOWING EXCHANGE TRANSFUSIONS OF BLOOD*

RICHARD H ANDRESEN CLARENCE W MONROE DOROTHY A MADDEN AND GEORGE M HASS

Previous studies of host graft interactions in adult rabbits showed that it was possible to distinguish between autologous and homologous musculo-fascial transplants by histologic methods[1] Although both types of trans plants underwent similar sequences of degeneration absorption and organization a characteristic inflammatory reaction was superimposed upon the newly formed vascularized connective tissue matrix invading the homo grafts This reaction was most conspicuous beneath the fascia of the grafts where a broad zone was created by the absorption of muscle fibers during the two weeks following transplantation (Fig 1) This inflam matory aspect of the host s reaction to a homograft was eliminated if the host and donor had previously been united in parabiosis for a few days[2] The host homograft interaction during the post parabiotic period was of two types which differed only in the degree of vascularization by the host (Fig 2) It was assumed that elimination of the inflammatory reaction

*From the Department of Pathology and Laboratory of Surgical Research Presbyterian Hospital Chicago Supported by grants from The National Heart Institute (H 1630 H 3915) United States Public Health Service The Muscular Dystrophy Associations of America Inc and The Otho S A Sprague Memorial Institute



Fig 1 This is a low power photomicrograph (X40) of an homologous musculofascial transplant 2 weeks of age The empty space above the tissue is the bursal space which normally forms over all types of musculofascial transplants The floor of the bursal space is formed by a pannus of vascularized connective tissue which has arisen from the host The pannus is firmly attached to the fascia of the transplant Beneath the fascia a broad zone has been created by the absorption of muscle fibers of the graft This musculofascial zone contains vascular channels and proliferating fibroblasts embedded in a collagenous matrix The zone appears dark due to the dense infiltration of lymphocytes It is in this region where the most distinctive differences between reactions to autografts and homografts of musculofascial tissue occur



Fig 2 This is a low power photomicrograph (X40) of a post parabiotic musculofascial cross homograft 2 weeks of age The floor of the bursal space consists of a delicate thin pannus of vascularized connective tissue arising from the host and firmly united to the fascia of the graft The fascia and region beneath it contain large patent vascular channels The muscle fibers of the graft remain in almost normal apposition to the overlying fascia In spite of the rich vascularization fibroblastic activity and collagen deposition are impaired Autolytic mechanisms involving skeletal muscle have been delayed There is no inflammation characteristic of the classical homologous incompatibility reactions

might be attributed to some change in the parabionts due to the cross circulation during parabiosis In order to make a preliminary test of this assumption, an experiment which involved nothing more than cross grafting between cross transfused rabbits was done This study eliminated all variables involved in parabiosis, except admixture of blood and this was controlled in quantity

METHOD

Twelve pairs of New Zealand rabbits were prepared for cross circulation under aseptic precautions and nembutal anesthesia Each animal was given heparin intravenously prior to establishment of the cross circulation In 2 pairs, the femoral arteries were cannulated with polyethylene tubing connected to a 3 way Luer syringe of 20 ml capacity Between 200 ml and 360 ml of arterial blood were totally exchanged between animals Protamine sulphate was given intravenously following completion of the exchange transfusions In 10 pairs under identical conditions, connections

with polyethylene tubing were established between the inferior vena cavae. Similar volumes of venous blood were exchanged between animals.

Ten to 28 days following the cross-transfusions, grafts of erector spinae muscle, measuring 2 x 1.5 x 0.5 cm. with attached fascia were resected under sterile precautions and cross-transplanted between paired cross-transfused animals. Control autografts were made at the same time. Two weeks following grafting each animal was sacrificed by injection of 1% procain solution into the cisterna magna. The animals were fixed in 10% formalin solution. Following fixation, the grafts and adjacent tissues were cut in serial blocks in a plane perpendicular to the spinal column. The blocks of tissue were prepared for microscopic study in paraffin sections and stained with hematoxylin and eosin.

RESULTS

Gross examination of the autologous and homologous transplants, 2 weeks of age, showed that they were well-healed in place. Bursal spaces had formed over both types of transplants. A thick pannus of red vascularized tissue had grown over the fascia of the autografts. The cross-homografts often were pearly white indicative of lesser degrees of vascularization of the pannus overlying the fascia.

Microscopic study of autografts, at 2 weeks of age, showed the customary orderly pattern of degeneration and organization without significant inflammation.

Microscopic study of cross-homografts showed a modification of the typical host-homograft interaction. This consisted of collagenous encapsulation of the transplant associated with a peculiar angiomatous vascular penetration which was most conspicuous beneath the fascia of the transplant. In spite of the rich vascularization, inflammation did not occur and fibroblastic proliferation with collagen deposition was meager. Autolytic mechanisms, normally very active in the subfascial zone of transplants, were curiously retarded. (Fig. 3.) This modification of the host-homograft interaction during the post-transfusion period was similar to that previously noted in post-parabiotic period. (Compare Figs. 2 and 3.) This change was readily reproducible between pairs of cross-transfused animals and seemed to bear no relation to different blood groups in rabbits.[3]

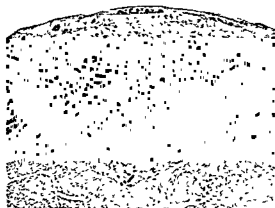


Fig. 3. This is a low-power photomicrograph (X40) of a post-transfusion cross-homograft, 2 weeks of age. The empty space above the transplant is the bursal sac. The floor of the sac consists of a richly vascularized pannus of host's tissue which has grown over the fascia of the transplant The vascular growth penetrates the fascia to terminate in the region beneath the fascia The associated sequences of healing have not proceeded to completion Note the absence of inflammation so characteristic of the host-homograft incompatibility interactions and, on the other hand, the similarity to post-parabiotic cross-homografts. Compare Figures 1, 2 and 3.

SUMMARY

Twelve pairs of adult rabbits were prepared for controlled transient cross circulation Amounts varying from 200 ml to 360 ml of whole blood were exchanged between pairs From 10 to 28 days following the single exchange of blood cross grafts of musculofascial tissues were made between cross transfused pairs of animals Autografts were simultaneously made Two weeks after grafting the grafts were prepared for miscroscopic study This showed the development of an angiomatous pattern of vascularization especially conspicuous beneath the fascia of cross homografts In spite of rich vascularization the usual sequences of healing observed in the auto grafts did not occur This post transfusion homograft reaction was identical to that seen in cross grafts between post parabionts Therefore it was pos sible to eliminate the inflammatory aspect of the host homograft interaction if massive exchange transfusions of blood were made prior to cross grafting

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SURGICAL ILLUSTRATION RESULTS OF EXPERIMENTATION*

JOHN H DICKSON

The past decade has witnessed an unprecedented advance in the effective ness and acceptance of teaching aids And in surgical fields this has been perhaps even more true than in many allied fields This has been due in large part to the nature of the material to be presented but also to the increased appreciation of surgeons of the advantages of well presented photographed material on a screen it is clearly visible to all whereas it may have been missed by the observer at the operation

Unfortunately many opportunities for proper recording of material of teaching value are lost either because no permanent record of any type is made or because the photographic record made is inadequate or is defective The purpose here is to emphasize certain points which are important in the construction of effective illustrative material Many or even most of these points are known to professional medical photographers but the majority of them may not be fully appreciated by the practicing surgeon – the one individual most in a position to secure effective teaching material

*From the Department of Medical Illustrations University of Mississippi Medical Center Jackson Mississippi

And by teaching material, I refer to photographs, slides of diagrams, and movies used at undergraduate, graduate, and postgraduate levels

DESIGN

As in the execution of an operation, illustrations must be planned to be effective It is an easy matter to achieve a desired effect if the objective has been carefully conceived and detailed in advance In this connection, let me note the value of preliminary consultation between the surgeon and the photographer Such items as target, features to be emphasized, lighting, coloring, and background must be considered in relation to each other – and with regard to the limitations of the photographic equipment available The members of our surgical staff and I hold numerous joint conferences before important commitments of time and money are made for particular projects Suggestions are exchanged and, in the case of movies, the operation is approached with both the photographer and the surgeon acutely aware of what can be expected to be shown They are ready to take advantage of any unanticipated opportunities to enhance the effectiveness of the film Above all, everyone appreciates the necessity of getting enough representative shots to maintain continuity for the viewer, with due allowances for cutting and editing Both surgeon and illustrator must constantly place themselves in the position of the audience and ask themselves what points they would most like to see

To summarize, then, good presentation must tell a story, and the essential elements of the story must be well understood and outlined before writing with the camera begins

HOW TO MAKE GOOD MOVIES

Everyone has admired excellent movies – and criticized poor ones – but perhaps the viewer is not always aware of why one film is pleasing and informative, while another drags and leaves little information with the audience Some of the points that must be adhered to will now be reviewed

Ideology. A physician would hardly begin to write a medical paper without first listing the major points to be emphasized He must have in mind what he wishes to convey and the order in which his message will unfold Precisely this same type of preparation is used in all effective movies and termed ideology A movie should tell a story in an orderly manner, with an introduction, a body, and an ending The educational content of a film will be no more and no less than the ideas built into it Again, when one prepares a lecture he outlines his ideas, and he considers the most effective means of putting these sequential ideas across to his audience So with the movie One common plan is to consider the general problem of, say, gallbladder disease in the first few minutes and then to particularize the major technical points with a cholecystectomy Neither the generalizations concerning gallbladder disease nor the mere execution of a cholecystectomy alone would achieve a satisfying intellectual depth and content but, taken together in sequence, they afford a balanced com position Particularly desirable are shots of a patient before coming to the operating room and as he leaves for home the successful case is thus

documented, and few things are more impressive to surgeons than the successful case.

Eye Appeal. Exposition should be clear, sufficiently simple and—one would hope—entertaining It is no longer taboo to present to physicians medical material in an attractive manner, and color photography has enormously broadened the opportunities for contrast

Tempo. The province wherein a movie excells the simple photograph is that of a tempo One can, of course, make a movie simply from colored photographs—stills, as it were—but the vital element of action or tempo would be lacking Of course, by means of multiple drawings to achieve *animation* the illusion of action can be produced, as in the Disney car toons, this can be employed quite effectively in medical movies None theless, the point I wish to emphasize here is that when one is performing an operation the camera should record movement, with size of field chang ing from scene to scene and length of scene determined by 1 action within scene, 2 length of subject matter, 3 general mood or tempo of the sequence in which the scene belongs

LENGTH	TIME (24 FRAMES/SECOND)	TEMPO
6	5/6 sec.	Choppy
1	1 2/3 sec	Short
2 to 3	3 1/3 sec	Medium
4 to 5	6 2/3 sec	Long
5 to 6	9 to 10 sec	Extra long—monotonous

Therefore a monotonous scene is broken by choosing a short and medium tempo using semi close up photography

Lighting. The angle of incidence equals the angle of reflection, there fore, if a light source is used at the lens axis reflection is directly back *to the lens so the principal source of illumination must come from a source* at an angle of approximately 45 degrees to be effective The source at lens must be relieved of intensity to alleviate the dominating reflectance

Editing Competent cutting or editing of the work print or original is equally important to effective movie presentation For example, the nar rator must decide how much time is allowed the total film length, and how many seconds each scene is to run The latter will be determined by the importance of the scene, the time required for visual comprehension and the number of words to be spoken (approximately 2 words per second) Time devoted to routine procedures well known to the audience, such as opening and closing the wound should be minimized

Editing and cutting (omitting ineffective scenes) presupposes that excess footage has been made it is far better to shoot' too much than too little

PREPARATION OF SLIDES

Slides are most often used to present diagrams, data narrative material and photographs Many of the same principles that have been described in connection with movie making also hold in connection with the prepara tion of slides, and these particular points need not be repeated

Perhaps the most vital single element in slide making is to limit the number of data or the complexity of the diagram presented. What the author may have spent weeks in digesting and preparing, the audience must grasp within a minute or so. It is useless to present a mass of data, or an excessive number of typewritten lines. While the electric typewriter — using all capitals and carbon paper backing — often produces acceptable copy, it is usually preferable to use a Leroy lettering set in order to achieve large and well spaced letters. A total of 4 lines of not more than 28 characters each is satisfactory, since the usual 3½ x 4 inch slide is longer in the transverse than in the vertical direction. Incidentally, movie screens have the same shape, and photographed material should fill the screen. Graphs should be simple, clearly labeled, and made of heavy lines. It is usually desirable to have a title on each slide.

Photographs of tumors and similar material should include some reference of measurement, perhaps a centimeter rule — for obviously a small tumor could be made to appear large if the camera were close to it. A dark blue background is a good one for tumor or organ photography. Photographs of chest x rays should include the P A and lateral views side by side.

Composition is important when slides are made for instructional purposes. For example, in a discussion of nutrition, one might set up a tray bearing jello, cottage cheese, spinach, a red apple, and other foods of vivid colors, with labels giving caloric or protein content and all against a blue background. In general, the more the realism achieved, the more valuable will the slide be as a teaching aid. For this reason, black and white slides of pathologic material have been replaced with color, which greatly enriches the surgical pathology experience of the medical student.

MEDICAL EXHIBITS

Medical exhibits are usually constructed to make a single point or to present a subject. They may consist of 8 x 10 Kodachrome transparency enlargements mounted in suitable cardboard frames. However, regardless of the photograph, in most instances it is useful to print identification of the lesion on the cardboard frame. Otherwise, many physicians will not be able to identify the tumor or lesion promptly and will not pause long at the exhibit. Black and white transparencies can be made, but they are much less striking than are colored exhibits. Again, design and arrangement of material in the exhibit is of vital importance — that is, the medical paper, the movie, or the exhibit must each convey the comprehensive message without which no amount of color or other props will provide intellectual content.

There are of course innumerable other types of exhibits, but basically the idea is offered in an arresting and informative manner. Lighting must be good, whether from transparency cases, overhead sources, or floodlights in front.

SUMMARY

The importance of effective visual aids in modern surgical teaching has been emphasized. Careful planning will render illustrative material both entertaining and informative.

PHYSIOLOGY OF THE PALATOPHARYNGEAL AREA FOLLOWING POSTERIOR PHARYNGEAL FLAP PALATORRHAPHY*

RICHARD C WEBSTER AND RICHARD J COFFEY

Palatopharyngeal incompetency may be defined as leakage of fluid or solid substances through the palatopharyngeal lumen Such leakage results from inadequate closure of the sphincter valve and has adverse effects on nasal, speech, swallowing, and respiratory physiology

In many patients, these physiologic disturbances have been eliminated or improved clinically by posterior pharyngeal flap palatorrhaphy This operation consists of the attachment to palatal tissues of a flap from the posterior pharyngeal wall A bridge of tissue is thus permanently created *running across the palatopharyngeal space from front to back and dividing* the lumen into two smaller passageways

The series reported here consist of 44 patients (male and female, ages 1½ to 60 years) with palatopharyngeal incompetency resulting from congenital clefts or insufficiency, trauma and infection, surgical removal of tumors, and neurological disorders producing paralysis Thirty nine have undergone posterior pharyngeal flap palatorrhaphy

Direct observation and measurement through the mouth, posterior rhinoscopy, and, at times, nasopharyngoscopy, show that passage shape is always changed and that total size of the two postoperative passageways is smaller than the one lumen existing before surgery Not only are the palatal and the lateral and posterior pharyngeal tissues closer together in the "open position" during nasal breathing but total lumen size is diminished by the very bulk of the tissue bridge crossing the space

Lateral x ray measurements have shown routinely that the posterosuperior surface of the soft palate is closer to the posterior pharyngeal wall after successful flap attachment

Posterosuperior motion of the soft palate, most workers agree, is caused mainly by contraction of the levator muscles However, some, basing their conclusions on lateral x ray studies alone have given the levators full or almost full credit for closure, at least in speech

To date, AP or PA x ray studies have produced little knowledge of the roles of other muscles in providing closure The remaining investigations reported here were carried out to elicit this information

Ability to open and close the palatopharyngeal sphincter valve was determined before and after surgery by rhinometric methods All but 2 of the 39 patients could breathe nasally In those who had no other openings between the mouth and nose, this proved that at least one of the postsurgical passageways in the palatopharyngeal region was open In this group, 22 patients were old enough to cooperate Rhinometry, with these patients blowing against increased oral air pressure and phonating test speech sounds showed no or less nasal air leakage after surgery than prior to it This plus clinical improvement, indicated some ability to close the palatopharyngeal passageways when desired

*From The Webster Clinic for Plastic and Reconstructive Surgery Brookline Mass

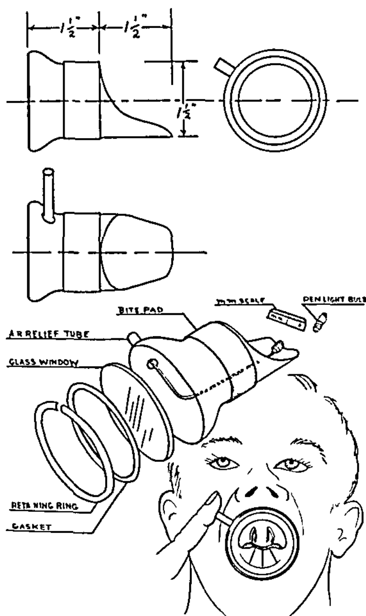


Fig 1 Palatopharyngoscope Left upper drawing shows palatopharyngoscope from side Beneath it is a view from above Right upper drawing shows palatopharyngoscope from front Beneath these is an exploded diagram These instruments may be used with or without the scale and light bulb The lowest view shows the instrument in place in the patient's mouth The finger is closing off the air relief tube

A random selection of 10 of this group was examined with palatopharyngoscopes (Fig 1) They were asked to inspire and expire orally and nasally, to repetitively phonate "Mom," "Pop," "Me," "Pea," to hiss, to blow against increased oral pressure, and to swallow small amounts of water High speed color cinematography allowed slow motion and frame by frame analyses of the movements of the structures

The soft palate rested lightly on the tongue in all cases during nasal breathing It moved posterosuperiorly to a slight to moderate degree and stayed in this elevated position during oral breathing in all patients Elevation was minimal to moderate on 'Mom" and a little greater on Pop in all It was rated slight to moderate on "Me" but in each patient was greater than on 'Mom' Some mesial motion of the palatopharyngeal muscles was noted in one patient in oral breathing and on all sounds but "Mom"

Pea elicited greater elevation of the soft palate in all cases than any of the tests mentioned so far Two patients showed moderate and one showed extreme mesial motion of the palatopharyngeal muscles on this sound The latter showed slight motion of the palatoglossal and superior constrictor areas and extreme mesial displacement of the salpingopharyngeal areas (Fig 2 A and B)

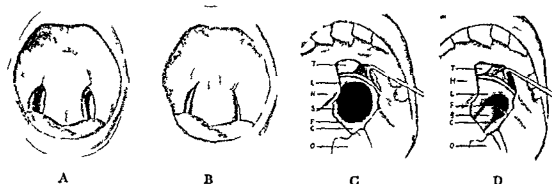


Fig 2 Drawings of palatopharyngeal regions taken from individual frames of high speed film strips A Nasal breathing The two passages are bounded by the pharyngeal flap medially and the palatopharyngeal muscle areas laterally B Same patient phonating Pea Palate elevated Salpingopharyngeal folds moving mesially behind palatopharyngeal muscles which are also moving toward the pharyngeal flap C Dental mirror inserted through hard palate perforation Black area is right passage in open position viewed from nasal side (Abbreviations T = turbinate L = levator muscle area H = hard palate S = salpingopharyngeal muscle area F = pharyngeal flap C = superior constrictor region O = oral side of distal portion of pharyngeal flap) D Reduction in size of passage as patient phonates Ah Note that the superior constrictor the salpingopharyngeal fold and the levator region all are contributing to the closure Marked mesial motion of the salpingopharyngeal muscle is evident

Hissing produced marked to extreme palatal elevation in all cases One showed moderate and 2 showed marked mesial displacement of the fauces One of the latter showed marked and the other showed extreme mesial motion of the salpingopharyngeal folds The former showed moderate elevation of the superior constrictor

Blowing against increased oral pressure produced extreme palatal elevation in 6 cases and marked in 4 Mesial motion of the lateral structures was less than on hissing in 2 and extreme in 8 patients

Observation of swallowing motions was difficult in 8 and impossible in 2 cases In 3 marked to extreme elevation of the palate could be seen at the beginning of the act In 5 palatal elevation and the beginning of mesial motion of the faucial tissues could be observed The tongue then interfered with vision However gagging produced extremes of motion of all muscles observed in all cases

Asking the patient to open the mouth widely and say Ah elicited more palatal elevation and mesial faucial and pharyngeal motions than did the production of other speech sounds observed through the palatopharyngoscopes

In other patients in our total series with large hard palatal openings observations of the nasal side of the palatopharyngeal area can be made

with the aid of a mirror inserted through the opening from the oral cavity (Fig. 2, C and D). These confirm previous findings.

CONCLUSIONS

Reduction in total palatopharyngeal lumen size produced by scar contraction and by the bulk of the flaps across the space tend to make more efficient the muscle motions effecting final degree of closure for a given patient.

Palatal elevation is the most important factor in most of these patients in providing competency and the improvement in nasal, speech, and respiratory physiology noted clinically. However, in some of the patients, motion of the palatopharyngeal, salpinopharyngeal, and superior constrictor muscles are also required. The component of palatopharyngeal closure which these muscles provide is particularly marked in gagging, in saying "Ah", and, we suspect, in swallowing.

CLINICAL STUDIES OF EXCRETION AND LOCALIZATION OF DIETHYL STILBESTROL DIPHOSPHATE LABELLED WITH RADIOACTIVE PHOSPHORUS (P^{32})

LESTER PERSKY, JACK S KROHMER AND JOHN P STORAASLI

Since the advent of I^{131} therapy for hyperthyroidism[1] and the extension of its use to carcinoma of the thyroid numerous attempts have been made to exploit the cancerocidal potential of radioactive isotopes in the neoplastic diseases of many body systems[2] Various methods of attaining this aim have been utilized including the administration of free isotopes and substances incorporating isotopes into their molecule

In urology the high level of prostatic acid phosphatase as compared to other tissues[3] seemed to afford an excellent tool to use in efforts directed toward delivering cytotoxic radioactivity to prostatic carcinoma In a previous communication[4] we described preliminary animal experiments in which studies were carried out with diethylstilbestrol diphosphate containing phosphorus 32 (Fig 1) The simple estrogen (stilfostrol) without radioactivity had proven to be effective in the palliation of carcinoma of the prostate[5] and this efficacy was felt to be due to its localization within the gland itself[6] A similar experiment incorporating human studies with a phosphoramide labelled with P^{32} was subsequently reported by Hummel *et al*[7] Their experience was similar to ours in that they concluded that there was slight and transient localization of radioactivity in the prostate Further study of labelled stilfostrol in humans seemed indicated however for a variety of reasons The chemical difference between stilfostrol and the phosphoramide employed by Hummel the differences in the prostates of humans and laboratory animals the known high serum acid phosphatase levels in patients with carcinoma of the prostate and the desire to learn something of the possible fate and metabolism of radioactive estrogens seemed to justify further observation

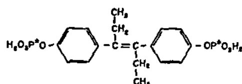


Fig 1 Molecular structure of diethylstilbestrol diphosphate showing position of P^{32}

*From the Department of Surgery Urological Service and the Department of Radiology Western Reserve University and the University Hospitals Cleveland Ohio Supported in part by the Miles Ames Research Laboratories Elkhart Indiana and conducted in part with facilities made available through contract number W 31 109 ENG 78 between the United States Atomic Energy Commission and Western Reserve University

METHOD

The patients in this study were all elderly males with carcinoma of the prostate diagnosed by the clinical findings, evidence of bony metastases, previous biopsy, or on the basis of elevated serum acid phosphatase. All patients were admitted to the urological service of University Hospitals of Cleveland. Two groups were studied. The experimental group of 5 men received diethylstilbestrol diphosphate labelled with phosphorus 32. A similar control group received inorganic phosphorus 32. In both series, the dosage of radioactivity was one millicurie. The amount of stilfostrol varied in the experimental group from 50 to 200 mg. because of the decay in radioactivity attending the relatively brief half-life of radioactive phosphorus. The compound in both groups was administered intravenously in 500 cc. of 5% dextrose.

Following injection, 24 hour urines were collected for 5 days. Specimens of blood were drawn at 1 hour, 3 hours, 6 hours and at 1, 2, and 3 day intervals. After 72 hours a transrectal biopsy of the prostate was taken, and a portion of bone and muscle was removed as well. Where possible, tumor-bearing bone as well as normal bone was removed.

All counts for radioactivity were made on dried or ashed samples using a thin-end window Geiger counter. The counts were corrected for background and were compared to an aliquot of the original dose. The counts were then expressed as a percentage of initial dose per gram based upon wet sample weight. The two groups were compared for possible differences in localization and excretion.

RESULTS

The average percentage of total dose per gram found on analysis of the tissue specimens in both is graphically represented in Figure 2. It can be readily seen that after 72 hours there was a greater percentage of initial radioactivity found in the prostate using inorganic phosphorus than with tagged stilfostrol. Muscle in both instances was very high in counts, but relatively speaking less so in the case of the P^{32} than in the case of stilfostrol. There was very little difference in radioactivity in bone when comparing the two substances.

The blood level curves of the two substances is seen in Figure 3. The rate of disappearance of the stilfostrol is somewhat slower than the P^{32} alone but parallels it in general configuration for the first 24 hours and

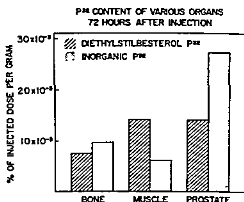


Fig 2. Percentage of initial dose of stilfostrol and inorganic P^{32} found in various tissues seventy-two hours after injection.

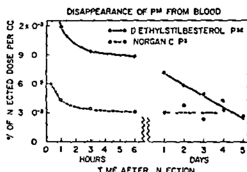


Fig 3 Blood disappearance curves of inorganic P^{32} and stilfostrol

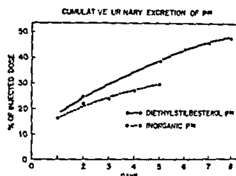


Fig 4 Cumulative urinary excretion patterns for inorganic P^{32} and tagged stilfostrol

then exceeds its disappearance rate Cumulative curves for urine are seen in Figure 4 The two substances again have the same general pattern of excretion with the stilfostrol appearing in the urine at a rate somewhat greater than the P^{32} alone

DISCUSSION

It is apparent from these data that just as in the case of the animal work little effective prostatic localization has been achieved by the administration of P^{32} labelled diethylstilbestrol diphosphate Our earlier work suggested that there were somewhat higher levels of radioactivity within the prostate in the very early period following injection The failure to demonstrate persistence of high activity in the prostate is probably a function of a high erosion factor to employ a term used by Hummel[7] and associates In the case then of the P^{32} which was formerly attached to stilfostrol the nature of the phosphate ion with its rapid integration into the larger overall body pool would not be expected to provide a long term deposit of radioactivity which would persist locally This aim therefore will have to be achieved by the testing of other substances

The high concentration of radioactivity in muscle found in humans is also consistent with previous animal experimentation This diffuseness of distribution also renders radioactive stilfostrol as a poor cancerocidal agent The curves of radioactivity in the blood and urine the slight differences of which are probably a function of the estrogen linkage in the experimental group similarly do not support the concept of effective localization Also the similarity to the distribution of inorganic phosphorus within the bone and muscle renders the likelihood of hematopoietic suppression a strong possibility since this is a known complication and hazard of phosphorus administration Preliminary studies with Carbon 14 labelled stilfostrol similarly do not corroborate organ specificity of stilfostrol We must conclude therefore that as yet we have no certain way of exploiting radioactivity in the treatment of carcinoma of the prostate other than by the direct injection which is currently being studied by Flocks[8] and other groups of investigators Perhaps a variety of substances exploiting different enzyme systems will ultimately need to be tested before an effective localizing agent is achieved which will deliver cancerocidal radiation upon parenteral administration

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THE EFFECT OF INCREASING PRESSURE IN THE RENAL VEINS AND OF OBSTRUCTION TO RENAL LYMPHATIC OUTFLOW UPON URINARY PROTEIN CONCENTRATION*

ROBERT KLAUS, JAMES SHALLOW, AND JOHN J MURPHY

An effort has been made to investigate the theories of the pathogenesis of benign postural proteinuria as proposed by Jehle,[1] Bull,[2] and Lowgren [3]

The investigation was divided into three parts In part one, the effect of increasing pressure in the vena cava upon the thoracic duct lymph flow, rate of urine formation, and urinary protein concentration was studied Part two was designed to determine the effect of obstruction of renal lymphatic outflow upon urinary protein concentration In part three, the effect of simultaneously increasing pressure in the inferior vena cava and renal lymphatic outflow obstruction upon urinary protein concentration was studied

PART I

Procedure. Twenty-eight experiments were performed upon adult female mongrel dogs, weighing between 18 and 49 pounds Anesthesia was produced by intravenous pentobarbital Animals which shivered during anesthesia, or whose respirations were not controllable with the respirator, were excluded

*From the Department of Surgery Division of Urology Hospital of the University of Pennsylvania, and the Harrison Department of Surgical Research Schools of Medicine, University of Pennsylvania Philadelphia

The animal was placed in the left dorsal decubitus position, anesthetized, and a cuffed endotracheal tube inserted This was connected to a Phillips and Bird respirator A catheter was placed in the bladder and allowed to drain constantly The right chest was entered through the tenth intercostal space and the thoracic duct and cisterna chyli visualized The thoracic duct was opened, a polyethylene catheter inserted and threaded to the cisterna where it was secured by two ligatures This catheter was brought out through the posterior angle of the incision and allowed to drain freely while the remainder of the preparation was accomplished

The dog was rotated into the dorsal decubitus position The femoral vein was exposed, opened, and a specially constructed double lumen catheter[4] (Fig 1) was inserted Proper placement of this catheter in the vena cava (just above the entrance of the renal veins) was accomplished by inserting the catheter until the balloon tip was palpable at the diaphragm through the chest incision and then withdrawing it 2 to 3 inches The balloon catheter was connected to a tuberculin syringe which was placed in a screw adjustment holder to permit accurate pressure control The polyethylene catheter was connected to a water manometer filled with a 2% heparin in 0 9% saline solution This solution was used to irrigate the catheter at intervals An intravenous infusion of 0 9% sodium chloride solution in a fore limb vein was maintained at a constant rate of flow of 2 to 3 ml /min throughout the remainder of the experiment

Control specimens of lymph and urine were collected during a period varying from 20 to 90 min At the end of this control period, the balloon was inflated in the vena cava until the desired venous pressure was reached and this pressure was maintained during the collection of lymph and urine samples In the first three experiments the venous pressure was allowed to return to normal before the next increase, but in all subsequent experiments increases in venous pressure were superimposed upon the previous pressure Both methods produced identical results insofar as protein concentration in the urine was concerned The urine was collected in chemically clean centrifuge tubes The bladder was expressed manually at the end of each urine collection period

Urine protein was measured by the Biuret method modified by *Gornall et al*[5] Purified bovine albumin was used as the standard, and the readings made on a Bausch and Lomb densitometer The urine was checked for microscopic hematuria in each experiment in which protein determinations were done, and samples containing more than five red blood cells per high power field were discarded

Results. There were 15 satisfactory preparations for measurement of lymph flow in the 28 dogs prepared as described above In 12, the rate of lymph flow increased significantly above control lymph flow rate when the pressure in the inferior vena cava was raised In 3, the increase was insignificant (Fig 2)



Fig 1 A simple double lumen balloon catheter 1—balloon catheter 2—polyethylene venous pressure catheter, 3—glass T tube 4—vaccine bottle cap 5—to tuberculin syringe 6—to venous pressure set

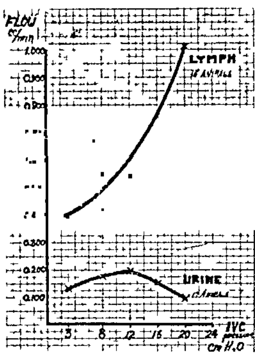


Fig 2 The effect of increased inferior vena cava pressure on the flow of lymph and urine

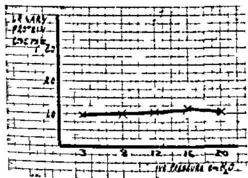


Fig 3 The effect of increasing inferior vena cava pressure upon urinary protein concentration Five animals

There were 17 satisfactory preparations for the determination of urine formation at varied inferior vena cava pressures In 12, there was a rise in urine formation until a pressure of 12 to 16 cm of water was reached in the inferior cava, followed by a decrease to below control rate as pressure in the vena cava was further increased In 4, there was a constant fall in rate of urine formation with increasing vena cava pressures In only one, the rate of urine formation rose as pressure in the inferior vena cava was increased

Determination of urinary protein concentration was performed in 7 animals In 5, there was no significant change in the urine protein concentration In 2, there was microscopic hematuria with high control urinary protein values, and there was no consistent change noted with increasing inferior vena cava pressures (Fig 3)

PART II

Procedure. The effect of obstruction to the lymph drainage of the kidney upon urinary protein concentration was studied in 6 dogs A control urine sample was obtained by catheterization of the bladder immediately following induction of anesthesia An endotracheal tube was introduced and connected to a Phillips and Bird respirator The right chest was entered and the thoracic duct ligated immediately above the cisterna chyli In two

animals the right renal pedicle was divested of all lymphatics in addition to ligation of the cisterna. In another animal, all branches of the lymphatics entering the cisterna chyli laterally and those bypassing the cisterna and entering the duct superiorly were ligated in addition to ligation of the thoracic duct. Urine sample were obtained at hourly intervals after ligation on all animals for several subsequent days on 2 dogs. Protein determination was determined as described above.[5]

Results. No significant alteration in urinary protein concentration was observed in these animals. The average control urinary protein concentration was 1.88 mg/cc. and the average urinary protein concentration following lymphatic obstruction was 2.00 mg/cc.

PART III

Procedure. In 3 animals simultaneous obstruction of renal lymphatic outflow and increasing inferior vena cava pressure was accomplished using the procedures described in Parts I and II. Urinary protein concentration was measured as before.[5]

Results. There was no significant change in urinary protein concentration in these animals. The average control urinary protein concentration was 1.18 mg/cc. and following the procedures was 1.06 mg/cc.

CONCLUSIONS

The experimental results of Part I indicate that increased pressure in the inferior vena cava above the renal veins results in:

1) An exponential rise in the rate of lymph flow.
2) A rise and then a fall in the rate of urine formation.
3) No change in the urinary protein concentration.

The last two conclusions confirm the work of Farber *et al.*[6] who performed similar experiments in man.

The data obtained in Part II indicate that simple obstruction of renal lymphatic outflow does not produce proteinuria.

The data obtained in Part III indicate that combined renal lymphatic outflow obstruction and increased inferior vena cava pressure above the renal veins does not produce proteinuria.

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THE EFFECT OF DIET ON THE RETURN OF FUNCTION OF KIDNEYS IN DOGS AFTER RELEASE OF URETERAL OBSTRUCTION FOR ONE WEEK*

WALTER S KERR, JR

It has been found that kidneys with ureteral obstruction of one week do not recover function (GFR and ERPF) to the same extent in the presence of the contralateral kidney as they do if the contralateral kidney is removed shortly after the ureteral obstruction is released [1] In an effort to determine the cause of this observation the effect of various amounts of protein in the diet on the rate and degree of recovery of function has been investigated After release of the obstructed ureter three levels of protein intake 1) low protein diet (2 1 gm protein/kg), 2) moderate protein diet (12 gm protein/kg), and 3) high protein diet (36 gm protein/kg, approximately 6 pounds of horse meat per day) were studied

METHOD

Trained female dogs of unknown age, 13 to 16 kg were used Control studies were made while all dogs were on a moderate protein diet Renal clearance tests were done on 2 or more different days without anesthesia in a 16 to 18 hour fasting state One thousand milliliters of water were given by stomach tube to effect diuresis Each renal clearance test represents 3 successive 10 minute collection periods and the average of these periods was taken as the clearance value

After control studies were complete, the right ureter was exposed under nembutal anesthesia, divided, and the ends ligated One week later, under nembutal or chloralose anesthesia, the proximal end of the ureter was exposed, the ureteral pressure, the pelvic capacity, and the total solute concentration were determined The ureter was then sutured to the skin Clearances were started, and one hour after release of the ureter, collections were started The right kidney urine was collected through a multi eyed No 8 catheter, and the left kidney urine through a No 16 catheter in the bladder The glomerular filtration rate (GFR) was estimated by the clearance of inulin [2] The effective renal plasma flow (ERPF) was estimated by the clearance of paraminohippurate [3] Sustaining doses of PAH and inulin were given in isotonic saline, 3 6 ml/min with a Bowman constant infusion pump All values are corrected to milliliters per minute per 1 m² of body surface area (BSA) [4] After release of the ureter, standard renal clearance tests were done at weekly intervals until maximum recovery† was noted Thereafter at least three standard renal clearances were made at intervals of 1 to 2 weeks and the studies were then terminated The freezing point depression was measured using a Fiske Associates osmometer Urine was collected 24 to 48 hours after dehydration and fasting to determine

†Maximum recovery—The day that the fraction contributed by each kidney became constant In the animals with contralateral nephrectomy the day of maximum recovery was the one that the clearances of the right kidney ceased increasing

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Table 1 Effect of Moderate and Low Protein Diet on the Degree of Recovery of GFR and ERPF in Dogs with Ureteral Obstruction Followed by Contralateral Nephrectomy

DOG	DIET: DURING CONTROL STUDIES	DIET: AFTER UNTYING RIGHT URETER	CONTROL GFR† ML/MIN	CONTROL ERPF† ML/MIN	DAY OF MAXIMUM RECOVERY: NO OF DAYS POST RELEASE OF RIGHT URETER	GFR CLEARANCE	% CONTROL	NO OF DAYS POST RELEASE OF RIGHT URETER	ERPF CLEARANCE	% CONTROL	NPN POST NEPHRECTOMY: HIGHEST	NPN POST NEPHRECTOMY: LOWEST
3	Moderate	Moderate	40††	103††		68†††	170	14	215	208	173	31
10	Moderate	Moderate	35††	177††	14	63	180	14	167	113	not done	not done
53	Moderate	Moderate	56	207	28	75	134	28	314	152	175	43
61	Moderate	Low	52	135	14	54	103	14	128	94	28	18
62	Moderate	Low	50	95	14	53	106	21	118	124	35	32

†Clearance/kidney calculated as preligation average total divided by 2

††Clearances done under nembutal anesthesia and saline diuresis (1000 ml 30 min prior to priming doses)

†††This figure is average of 16 clearances done 21 to 647 days after rel

concentrating ability Nonprotein nitrogen was determined by the method given in the *John Laboratory Manual*, 1929 edition

Two days after the release of the right ureter, the left kidney was removed in 5 dogs

RESULTS

A Comparison of Effect of Moderate Protein Diet and Low Protein Diet on Function in Dogs Obstructed One Week Control Kidney Removed Table I shows that 3 dogs kept on a moderate protein diet after the release of the right ureter, the GFR and ERPF reached higher levels (% control GFR and % control ERPF) than the 2 dogs maintained on the low protein diet Seven weeks after ureteral release the protein intake of the latter 2 dogs increased from the low protein to the moderate protein diet with a subsequent increase in GFR and ERPF The NPN after nephrectomy was measured in 4 of these 5 animals It will be noted in Table I that in 2 of the animals maintained on a moderate protein diet, the NPN increased to 173 and 175 mg % and ultimately dropped to 31 and 43 mg % In the 2 dogs on a low protein diet after nephrectomy, the NPN did not become elevated above control levels

B Effect of High Protein Diet and Moderate Protein Diet on Function Control Kidney Intact In view of the fact that a moderate protein diet caused greater return of function of the previously obstructed kidney than the low protein diet in animals with the control kidney removed a high protein diet to another group of animals with control kidney intact, was given to determine whether still greater recovery could be effected or to determine whether the previously obstructed kidney would be injured as a result of an excessive protein intake† as has been suggested by Addis[5] It was hoped that the high protein diet†† would tax the excretory capacity of the 2 kidneys maximally

The high protein diet was administered by forced tube feedings of blenderized cooked horse meat (36 gm protein as 180 gm of horse meat/kg/day)††† in 3 divided doses every 8 hours This high protein diet was successfully carried out in 2 dogs (No 73 and No 75) for 8 weeks †††† The renal clearance tests during the control and postoperative studies were done in the following manner There were three successive periods Period A three 10 min collections were made after 45 min equilibration in a state of 16 to 18 hour fasting water available Period B 1000 ml of water were administered by stomach tube 45 min equilibration and three 10 minute collection periods Period C isotonic saline 10 ml /min administered intravenously during 45 min equilibration period 500 ml water were given by

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It is recognized that there is a difference in the damaged kidneys as discussed by Addis and those presented here

**Attempts to give more than approximately 6 lbs of meat per day were not successful only a few dogs would tolerate such feedings for more than a few days

† The amount is based on the weight at the time of the release of the ureter The amount was not increased as the weight of the dog increased as a result of this large intake

††**Dogs with contralateral nephrectomy were not fed more than 12 mgs protein/kilo/day This resulted in the elevation of NPN to 175 mgms % without interfering with general appearance behavior or appetite More than 12 gms protein/kilo would almost certainly have pushed the NPN over 200 mgms % Above this figure dogs become lethargic and refuse to take anything p o except water and small amounts of meat or milk

Table 2 Comparison of Effect of High Protein Diet and Moderate Protein Diet on GFR and ERPF R/L % in Dogs After Release of Ureteral Obstruction for 7 Days – Control Kidney Intact

DOG	DIET		CONTROL GFR† ML/MIN			CONTROL ERPF† ML/MIN			GFR R/L % AVERAGE OF 3 OR MORE CLEARANCES 21-49 DAYS AFTER RELEASE OF URETER††			ERPF R/L % AVERAGE OF 3 OR MORE CLEARANCES 21-49 DAYS AFTER RELEASE OF URETER††		
	DURING CONTROL STUDIES	AFTER UNTYING RIGHT URETER	PERIOD			PERIOD			PERIOD			PERIOD		
			A	B	C	A	B	C	A	B	C	A	B	C
73	Moderate	High	40	47	54	130	171	203	74	77	87	77	81	89
75	Moderate	High	52	62	71	142	165	224	65	81	91	69	80	86
1 4,6 12 13	Moderate	Moderate							53†††			50†††		

†Clearance/kidney calculated as preligation total average divided by 2 Three control studies were done in dogs 73 and 75

††There was only a slight increase in values after 21 days after release

†††Average of these 5 dogs on the Day of Maximum Recovery Control and post release studies were done under nembutal anesthesia and saline diuresis

stomach tube 15 min after the saline infusion was started 3 collection periods of 5 to 7 min were made

(1) Comparison of GFR and ERPF Five dogs 1, 4 6 12 13 (Table 2) were maintained on a moderate protein diet before and after ureteral obstruction The control and post release studies on these dogs were done under nembutal anesthesia and 800 ml of isotonic saline to cause diuresis The flow of urine in these animals was approximately the same as it was in dogs No 73 and No 75 in period A and thus the values obtained in Period A are used for comparison The average GFR R/L and ERPF R/L of the 5 dogs on the moderate protein diet was 53% and 50% respectively on the day of maximum recovery In contrast the average GFR R/L and ERPF R/L in the 2 dogs on the high protein diet were distinctly higher than in those animals receiving the moderate protein diet † Hence there seemed to be no evidence that the high protein diet damaged either kidney nor was there evidence that the high protein diet interfered with the rapidity of recovery

(2) Effect of Hydration and of Isotonic Saline Load on GFR and ERPF in Dogs on a High Protein Diet After Maximum Recovery It had been hoped that the high protein diet would provide sufficient stimulus so that the excretory capacity of both kidneys would be maximally stimulated Table 3 shows that neither the previously obstructed kidney nor the control kidney was working at maximum capacity for right GFR and ERPF and left GFR and ERPF in period A were less than the values in Period B Right GFR and ERPF showed a further increase in Period C Left GFR and ERPF in Periods B and C were comparable The clearance tests included in the above data were done 16 hours after the last feeding On 2 days clearance tests were done 8 hours after the last feeding and results comparable to the above data were noted Thus 8 hours after approximately 2 pounds of horse meat neither kidney was maximally stimulated

(3) Level of NPN During High Protein Feeding The control values for nonprotein nitrogen in dogs No 73 and No 75 were between 20 and 30 mg % Following maximum recovery the nonprotein nitrogen 8 hours after the last feeding was between 40 to 50 mg % The nonprotein nitrogen 7 days after decreasing the diet to a moderate protein diet was back to control levels or less

(4) Effect of High Protein Diet on Ability to Concentrate and Dilute Urine The ability to concentrate and dilute urine was measured following maximum recovery Urine was collected following a 24 hour period of dehydration and the concentration was measured The ability of the previously obstructed kidney to concentrate urine was impaired to approximately the same degree noted in animals receiving a moderate and a low protein diet The ability to dilute urine was not impaired

(5) Solute Excretion by the Right and Left Kidney In addition to failure to recover its ability to concentrate urine as well as the control kidney the previously obstructed kidney also was damaged in respect to handling solute Under circumstances of solute loading (Period C) more solute (15 to 80%) was excreted by the previously obstructed kidney than by the control

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†In one dog who received 18 24 gms of protein/kilo less recovery was noted than in dogs 73 and 75

Table 3 Effect of Hydration and of Isotonic Saline Load on GFR and ERPF After Maximum Recovery in Dogs 73 and 75

DOG	DIET: DURING CONTROL STUDIES	DIET: AFTER UNTYING RIGHT URETER	CONTROL GFR† PERIOD A	CONTROL GFR† PERIOD B	CONTROL GFR† PERIOD C	CONTROL ERPF PERIOD A	CONTROL ERPF PERIOD B	CONTROL ERPF PERIOD C	RIGHT GFR PERIOD A	RIGHT GFR PERIOD B	RIGHT GFR PERIOD C	LEFT GFR PERIOD A	LEFT GFR PERIOD B	LEFT GFR PERIOD C	RIGHT ERPF PERIOD A	RIGHT ERPF PERIOD B	RIGHT ERPF PERIOD C	LEFT ERPF PERIOD A	LEFT ERPF PERIOD B	LEFT ERPF PERIOD C
									AVERAGE OF 3 OR MORE CLEARANCES 21 49 DAYS AFTER RELEASE OF RIGHT URETER											
73	Moderate	High	40††	43	54	130	171	204	47	51	61	59	67	67	154	187	196	209	216	216
75	Moderate	High	52††	63	72	142	165	224	41	58	62	49	66	67	129	163	182	186	206	212

†ml /min

††Clearance/kidney calculated as preligation average total divided by 2

DISCUSSION

Whether the degree of recovery (GFR and ERPF) in the dogs with contra lateral nephrectomy on a moderate protein diet was independent of the diet was investigated by giving similar preparations and a low protein diet GFR and ERPF after maximum recovery in those dogs on the low protein diet was definitely lower than in animals on the moderate protein diet Four to 6 weeks after maximum recovery was noted in the dogs on the low protein diet they were given a moderate protein diet and their clearances showed an increase (about 20%) at the end of one week

Following a period of ureteral obstruction recovery is better if the control kidney is removed This finding could be due either to an inhibitory action of the remaining normal kidney or to a lesser stimulus for recovery in the presence of a normal kidney The fact that a sufficiently high protein intake increased the renal clearances of the previously obstructed kidney in the preparation with the intact contralateral to the same levels as those observed for the previously obstructed kidney in the absence of a normal contralateral indicates that a lack of stimulation rather than inhibition is the important factor

The high protein diet did not have the desired effect of producing maximum recovery of clearances (GFR and ERPF) in dogs No 73 and No 75 as had been hoped for the GFR and ERPF in Period C was greater than in Period B which in turn was greater than Period A Even in Period C the GFR and ERPF R/L % reached only 85 90% † It is possible that a greater protein intake might have resulted in further recovery of function on the part of the previously obstructed kidney and that complete recovery (R/L 100%) might have been achieved

It has been noted in a prior publication that the ability to concentrate urine on the previously obstructed side in dogs on a moderate protein diet was reduced to 50 to 70% R/L This degree of depressed concentrating ability persisted in dogs on a high protein diet and also on dogs on a low protein diet During saline infusion (Period C) a higher rate of solute excretion from the previously obstructed kidney with a lower GFR than from the normal is probably the result of some residual tubular damage

SUMMARY

The effect of a low moderate and high protein diet on the degree of return of function (GFR and ERPF) of kidneys after ureteral obstruction of seven days has been evaluated

A greater degree of function is noted with an increased intake of protein

There was no evidence that a high protein diet approximately 6 pounds of horse meat per day per dog damaged the previously obstructed kidney or interfered with its immediate or subsequent recovery

† A study of the data comparing left C_{IN} and C_{PAH} in dogs 73 and 75 to values obtained in control studies does not suggest that the high protein diet stimulated the left kidney to increase its function above the controls Dog 45 control GFR Period C was 54 Two clearances done 3 hours after 2 lbs of meat p.o—GFR period C was $\frac{148}{2} = 74$

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THE EFFECT OF INCREASING PRESSURE IN THE BLADDER AND COLON UPON THE FORMATION OF URINE AND RENAL LYMPH*

M K MYINT AND JOHN J MURPHY

In the course of some experiments concerning the effect of diuresis upon the rate of renal lymph flow a direct relationship was noted between the degree of bladder distention and the rate of renal lymph formation[1] (Fig 1) Increasing intravesical pressure has been reported to result in decreased urine formation [2] This phenomenon occurs whether or not the ureters are in continuity with the bladder The exact mechanism by which this effect is obtained is not clear The experiments to be described were designed to determine the effect of bladder distention upon the rate of urine formation as well as renal lymph flow and to elucidate the mechanisms by which these effects are accomplished

Adult mongrel dogs were used as experimental animals They were anesthetized with intravenous pentobarbital The trachea was intubated with

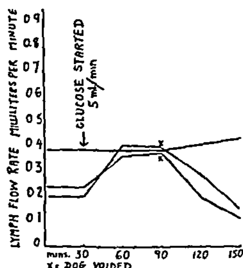


Fig 1 Effect of increasing intravesical pressure (ureters intact) upon rate of lymph flow

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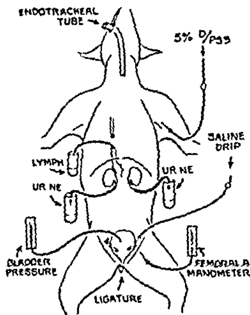


Fig 2 Diagram of experimental arrangement

a cuffed endotracheal tube which was connected with an automatic respirator The cisterna chyli was cannulated by means of a polyethylene catheter introduced through the thoracic duct in the right chest The ureters were divided near the bladder and catheterized with polyethylene catheters so that the urine output could be measured Blood pressure was measured by means of a mercury manometer connected to a cannula in the femoral artery Five per cent dextrose in saline solution was administered intravenously at a constant rate of 80 drops per minute for one hour (Fig 2) Control collections of lymph and urine were made until the flow was standardized for two collection periods This usually required approximately one half hour

In the first group of animals the urethra was ligated A tube was introduced into the bladder and connected to a water manometer Through this system the bladder pressure was increased to 60 cm of water pressure in increments of 20 cm Each new pressure level was maintained for ten minutes After reaching 60 cm and maintaining it for 10 minutes the pressure was returned to zero Collections of lymph and urine were made during each 10 minute period

In the second group of animals the bladder pressure was kept at zero while the pressure in an isolated segment of colon was similarly raised and lowered Rates of urine formation and lymph flow were measured continuously in each group

RESULTS

Animals whose blood pressure failed to remain at a constant level were discarded Seven dogs were included in the first group of animals (effect of increasing bladder pressure) A representative graph of such an experiment (Fig 3) shows that the rate of urine flow decreases as the bladder pressure increases When the bladder pressure returned to zero the urinary output leveled off or showed a tendency to return to control levels Changes in rates of lymph flow were inconsistent and probably not significant

In the animals in group 2 (increased intracolonic pressure) 3 animals

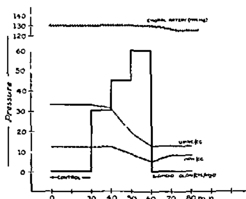


Fig 3 Effect of increasing intravesical pressure upon rates of urine and lymph formation (ureters severed)

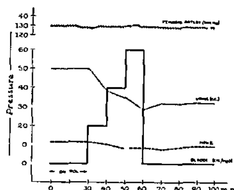


Fig 4 Effect of increasing intracolonic pressure (ureters severed)

met the experimental criteria A representative graph from one of these experiments (Fig 4) demonstrates that the rate of urine formation diminished as the pressure within the sigmoid colon increased The rate of lymph flow decreased slightly in 2 dogs and increased slightly in one

These experimental results were interpreted as indicating that the oliguria produced by distention of the bladder is probably mediated through the autonomic nervous system since it also occurs with distention of the sigmoid colon Distention of these hollow viscera has little effect upon the rate of renal lymph formation when the ureters are detached from the bladder, suggesting that the increased rate of lymph flow noted when the bladder is distended while the ureters are intact is probably due to some mechanical effect exerted upon the kidney by this distention, or to nervous stimuli transmitted through the ureteral nerves

Experiments with parasympathetic blocking agents tend to confirm the impression that these phenomena are mediated through the autonomic nervous system as shown in this graph (Fig 5)

SUMMARY

Distention of the bladder and the sigmoid colon produces oliguria, probably by reflex activity upon the kidney mediated through the autonomic nervous system

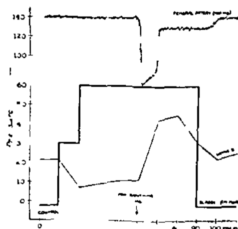


Fig 5 Effect of parasympatheticolytic agent

Increased pressures in the bladder and sigmoid colon when the ureters were detached from the bladder produced no significant changes in the rate of renal lymph formation This suggests that the increased rate of renal lymph formation noted when the ureters are attached to a distended bladder is due to some mechanical effect (direct hydrostatic or lymphatic) or to a reflex mediated through the ureteral nerves

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AN EVALUATION OF THE ROLE OF THE ARTIFICIAL KIDNEY IN THE TREATMENT OF ACUTE RENAL FAILURE*

JULIAN S ANSELL, MILTON P REISER, ROBERT ABERNATHY, DENNIS KANE, HARRIS HYMAN III, AND ARNOLD KOLODNY

Most groups now report mortalities around 60% in patients with acute renal failure requiring treatment by extra corporeal dialysis[1 2 3 4] indicating that earlier optimistic predictions about results of such treatment were premature (A notable exception are the results of Alwall *et al*[5] from Sweden who reports a mortality of 35%[6]) The types of acute renal failure most likely to benefit from dialysis have not been fully classified An analysis of the results of treatment of 25 consecutive cases at the University of Minnesota Hospitals from 1952 to 1956 provides a spectrum which helps to identify the categories of patients most likely to benefit from such therapy

Dialysis was carried out in each instance as an adjunct to conservative therapy Only when all other available means of treatment were exhausted and the patient's condition continued to deteriorate did we resort to extra corporeal dialysis In a few cases peritoneal lavage was tried first The Brigham modification of the Kolff artificial kidney manufactured by Edwin A Olson of Ashland, Massachusetts, was used in all cases[6] The 25 cases are divided into 5 etiological groups

METHOD

GROUP 1 included 9 cases of renal failure following extensive surgery with complications One patient had two-thirds of the small bowel resected, a second suffered cellulitis of the abdominal wall, peritonitis and retro coecal abscess following cecostomy for ileus after a herniorrhaphy The

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third case had abscess of the lesser bursa following perforation of a duodenal ulcer There were two cases of anuria following rupture of an abdominal aneurysm and replacement grafts One patient was oliguric because she received mismatched blood during radical mastectomy One patient developed nephrosis following a transurethral resection during which distilled water was used as irrigating fluid Two patients had nephrosis following abdominoperineal resections for carcinoma of the rectum Our efforts with this group proved only temporarily beneficial None of the 9 treated survived

GROUP 2 included three patients with subacute glomerulonephritis and one with diffuse fibrinoid disease Of the four a 13 year old girl with subacute glomerulonephritis whose diagnosis was ascertained by needle biopsy of the kidney remained oliguric 28 days then recovered following use of the artificial kidney peritoneal lavage and cortisone therapy

GROUP 3 contained 3 patients with nephrosis following accidental trauma and one patient with septic abortion One of these trauma victims recovered after 2 dialyses and another might have recovered if a more radical debridement of injuries including amputation of a crushed leg had been carried out

GROUP 4 contained patients with acute renal failure secondary to exposure to toxic drugs Two inhaled carbon tetrachloride one drank ethylene glycol and a fourth ingested large quantities of barbituates in a suicidal attempt Of these patients only the patient who ingested ethylene glycol failed to recover He required 3 dialyses lost 24 pounds of weight in 27 days but was found to have marked pulmonary edema in addition to confluent bronchopneumonia at autopsy Pulmonary edema and infection are two of the commonest fatal complications of this disease More stringent fluid restriction might have saved this patient s life

GROUP 5 was made up of 4 patients whose cesarean deliveries were complicated by incompatible blood transfusions with ensuing oliguria or anuria Of the 4 one patient died after 12 days of oliguria the urinary output was 1350 cc 24 hours prior to expiration Post mortem findings included massive necrosis of the pituitary secondary to infarction and hemolytic tubular disease of the kidneys A second dialysis was indicated here in spite of the diuresis and probably hormone replacement therapy as well

Table 1 summarizes the results by groups

Table 1

GROUP		TREATED	ALIVE
1	Renal failure complicating extensive surgery	9	0
2	Glomerulonephritis and fibrinoid disease	4	1
3	Nephrosis following trauma	4	1
4	Carbon tetrachloride toxicity ethylene glycol and barbituate poisoning	4	3
5	Caesarean section with transfusion reaction	4	3

DISCUSSION

Results were poorest in patients with extensive surgery and complications (Group 1). We intend to continue to try to salvage such individuals with dialysis, but feel that a fresh *preventive* approach is indicated in these patients Such measures might include hydration of the patient intravenously preceding surgery to maintain a protective diuresis during the operative period. Whenever, in the course of surgery, prolonged shock occurs or transfusion reaction is suspected, temporary denervation of the kidneys by injection of long-lasting locally acting anesthetic agent around the renal pedicle is suggested, if at all feasible. The outcome in patients with connective tissue disease (Group 2) depends upon resolution of the underlying disease process More aggressive treatment of the victims of trauma to include radical debridement of injured tissues is indicated (Group 3). We must not be guilty of saving a leg only to lose the man. The results of dialysis in those patients suffering from toxic nephrosis, barbiturate poisoning, (Group 4), and transfusion reaction complicating deliveries (Group 5) is most gratifying.

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EXCRETION OF FACTORS CONCERNED IN THE FORMATION OF URINARY CALCIUM CALCULI AFTER THE ADMINISTRATION OF ACETYLSALICYLIC ACID AND GLUCORONOLACTONE†*

FRANK C. HAMM, SIDNEY R WEINBERG, DAVID KARANSKY,
LEO KESNER, AND PHILIP LEWIS

Though the etiology and pathogenesis of primary calcium urinary stones have not been determined, one factor, that of an increased amount of calcium in urine appears implicated in many instances However the

†Figures showing the experimental data will be published later in a more detailed report of this work

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amount of calcium that urine can maintain in solution without precipitation or its solubility is unknown[1] It is generally believed that urine is supersaturated with calcium as determined by equilibration experiments in which urine of known calcium content is agitated with excess calcium phosphate If precipitation occurs calcium goes out of solution and a supersaturated state existed if some of the excess calcium phosphate goes into solution then undersaturation probably preexisted Flochs[2] and Andrew[3] found supersaturation common in the urines of stone forming patients

The physical state of calcium in urine has a direct hearing on its solubility It can be assumed that calcium in urine is either present as free ions in equilibrium with calcium phosphate or held in unionized organic compounds by union with citrates or glucuronides[4,5] It is obvious that the greater amount of calcium held in organic union the less free calcium ions will be available for precipitation and presumably for calculus formation

With this in mind Prien and Walker[6] have drawn attention to the possible solubilizing effects of glucuronides on calcium in urine They have recommended the administration of acetylsalicylic acid or salicylamide as drugs that can forward the formation of calicum glucuronide chelated organic unions similar to the calicum citrate complex and thus prevent the formation and growth of urinary tract calculi This premise is based on experiments by Newberg and Grauer[7] who showed that the presence of menthol glucuronide in an *in vitro* solution of Ca Cl and Na H_2 PO can prevent calcium precipitation However a 0 139 Molar solution of menthol glucuronides containing 46 gm /L was used a concentration that cannot be obtained in physiologic solutions Cessi s[8] work which followed the studies of Newberg and Grauer may also have bearing on the solubility of calcium salts in urine Cessi s experiments were done with isolated liver slices and demonstrated absorption of tagged Ca_3 $(PO_4)2$ from physiologic saline solution by the liver slices through the action of the glucuronides Neutral or slightly alkaline tissue pH is necessary for the formation of an adequate amount of the enzyme glucuronidase which acts as a catalyst for the reaction If an analogy can be drawn between the reactions in the liver slices and those in the kidney then the administration of acetylsalicylic acid and glucuronolactone which leads to an increased amount of glucuronides in the urine and presumably in the tissues might alter the concentration or the state of calcium in plasma and perhaps the clearance of calcium If so perhaps subjects to whom acetylsalicylic acid salicylamide or glucuronolactone was administered might have different amounts of calcium in their urine than before the administration of these drugs To test this theory determinations of the factors involved in the *solubility* of calcium were done before and after the administration of acetylsalicylic acid and glucuronolactone in both normal individuals and in those patients who had formed stones The determinations were performed on individuals of varying race age sex and pregnant women It was hoped that a range of normal variation for comparison would further delineate these values in stone forming patients

METHOD

Twenty six patients, of whom 12 were normal individuals, 6 were pregnant females and 8 were stone formers, were studied as follows

Each subject had 24 hour urines collected on the first 2 days of the experiments as controls On the third and fourth days each experimentee was given 6 gm of glucuronolactone per day and had 24 hour urines collected on the fourth and fifth days On the sixth, seventh, and eighth days each individual was then given 2 gm of acetylsalicylic acid per day and had 24 hour urines collected on the seventh and eighth days The subjects were on unrestricted diets Surface tension, specific gravity and pH determinations were done on uncentrifuged urine Determinations of the calcium, phosphorus, citric acid, and glucuronide content of the urines were done on centrifuged aliquot samples obtained from the 24 hour specimen, which had been preserved with toluene It had been determined in our laboratory by 11 experiments that in uninfected urine the calcium and phosphorus content of both uncentrifuged urine and urine centrifuged for 5 min at 1500 rpm is within the same range of results suggesting that any calcium phosphate not in solution remains in suspension in the urine and does not precipitate at room temperature

Specific gravity was measured by a urinometer Surface tension was measured by means of a Revici urotensiometer [9] Although this instrument does not accurately measure surface tension, it is a rapid method, useful for comparing groups of urines, i e, urines from normal individuals as compared with stone formers The pH was read on a Beckman model pH meter Calcium was determined by means of the flame photometer attachment of the Beckman spectrometer [10] Phosphorus was determined by the method of Fiske and Subba Row [11] Citrates were determined by the method of Pucher[12] and glucuronic acid was determined by the Tollens napthoresorcinol test as modified by Fishman [13]

In addition equilibration experiments were done to determine whether any alteration of calcium solubility takes place after the administration of the drugs due to any possible increase in content of citrate or glucoronisde in the urine In these experiments 50 cc of urine of known calcium content was equilibrated with calcium phosphate for 2 hours following which a similar sample was equilibrated with the same amount of calcium phosphate plus a 0 003 Molar (1 0 gm /L) solution of menthol glucuronide to determine if the drug *per se* could alter the solubility of calcium in urine These experiments were conducted at the pH of the urine as found The 1 gm /liter concentration of glucuronide was decided upon as this concentration was the highest physiologic level of the glucuronides as found in over 200 determinations in the previous experiments [14]

In a similar series of experiments 250 cc urine was equilibrated with calcium phosphate plus 0 1 gm of acetylsalicylic acid This experiment was decided upon as 20% of acetylsalicylic acid is excreted unchanged in urine while the remainder is altered to glucuronic acid [15]

RESULTS

The results were tabulated for each patient and the individual results were then averaged Our conclusions are as follows *(1) pH* pH values

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With this in mind, Prien and Walker[6] have drawn attention to the possible solubilizing effects of glucuronides on calcium in urine They have recommended the administration of acetylsalicylic acid or salicylamide as drugs that can forward the formation of calicum glucuronide chelated organic unions similar to the calicum citrate complex and thus prevent the formation and growth of urinary tract calculi This premise is based on experiments by Newberg and Grauer[7] who showed that the presence of menthol glucuronide in an *in vitro* solution of $Ca\ Cl_2$ and $Na\ H_2\ PO_4$ can prevent calcium precipitation However a 0 139 Molar solution of menthol glucuronides containing 46 gm /L was used, a concentration that cannot be obtained in physiologic solutions Cessi s[8] work which followed the studies of Newberg and Grauer may also have bearing on the solubility of calcium salts in urine Cessi s experiments were done with isolated liver slices and demonstrated absorption of tagged $Ca_3\ (PO_4)2$ from physiologic saline solution by the liver slices through the action of the glucuronides Neutral or slightly alkaline tissue pH is necessary for the formation of an adequate amount of the enzyme glucuronidase which acts as a catalyst for the reaction If an analogy can be drawn between the reactions in the liver slices and those in the kidney then the administration of acetylsalicylic acid and glucuronolactone which leads to an increased amount of glucuronides in the urine and presumably in the tissues might alter the concentration or the state of calcium in plasma and perhaps the clearance of calcium If so perhaps subjects to whom acetylsalicylic acid, salicylamide or glucuronolactone was administered might have different amounts of calcium in their urine than before the administration of these drugs To test this theory determinations of the factors involved in the solubility of calcium were done before and after the administration of acetylsalicylic acid and glucuronolactone in both normal individuals and in those patients who had formed stones The determinations were performed on individuals of varying race, age sex and pregnant women It was hoped that a range of normal variation for comparison would further delineate these values in stone forming patients

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RESULTS

The results were tabulated for each patient and the individual results were then averaged Our conclusions are as follows *(1) pH* pH ---

of urine do not change after the administration of the drugs used (2) *Surface Tension* This factor is an expression of the physical state of urine as related to the colloid crystalloid balance Differences in the surface tensions of urines obtained from stone formers and those of normal patients have been reported [16] Our studies do not indicate any such difference There was no alteration of the surface tension after the administration of acetylsalicylic acid or glucuronolactone in the three classes of subjects tested (3) *Concentration of calcium and phosphorus* The concentration of free calcium ions multiplied by the concentration of phosphate ions determines the critical level in urine for precipitation of calcium phosphate Our results indicate that the administration of acetylsalicylic acid or glucuronolactone did not increase either the total excretion or the concentration of calcium and phosphorus per milliliter beyond the range of values that existed before the administration of the drugs (4) *Concentration of citrate and glucuronide* As discussed the concentration of organic acids such as citrates or glucuronides in urine also has a direct bearing on the solubility product of calcium phosphate Vermeulen[4] has demonstrated that calcium held in organic union cannot migrate or dialyze

Although the citrates were elevated in some instances after the administration of the drugs used this was no general effect since in nearly as many instances the citrate levels were diminished

However the administration of glucuronolactone and acetylsalicylic acid in all instances raised the concentration of the glucuronides in urine but the equilibration experiments as described did not demonstrate any direct solubilizing action of the glucuronides on the calcium in urine at pH 6 the physiologic concentration used

SUMMARY

The administration of acetylsalicylic acid and glucuronolactone does not alter the excretion of calcium or any of the factors concerned with its solubility in urine

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THE PROTECTIVE EFFECT OF KIDNEY HYPOTHERMIA ON TOTAL RENAL ISCHEMIA*

PAUL R SCHLOERB RICHARD D WALDORF AND JOHN S WELSH

The protective effect of general hypothermia on renal vascular occlusion has been established[1] Preservation of kidney function for periods up to 2 hours of renal ischemia by local cooling to 20 to 25°C has been documented[2] Tissue metabolism and oxygen requirement should be decreased in proportion to the lowering of temperature thereby prolonging the permissible period of ischemia It is the purpose of this paper to present an experimental evaluation of this hypothesis

METHOD

Thirty four male and female dogs varying in weight from 15 to 22 kg were used for this study employing sodium pentobarbital anesthesia (30 mg/kg) with supplementary amounts as necessary The protective effect of cooling *in situ* on renal ischemia was studied in 15 dogs After a right nephrectomy the left kidney was freed up and the renal artery, vein and ureter were isolated and clamped with atraumatic arterial bulldog clamps after injection of 20 mg of heparin into the renal artery Following this the kidney was placed in a sterile polyethylene bag with inversion of the bottom of the sac so that the kidney would be cooled to 2 to 4°C but would not be in direct contact with ice water placed in the bag Occlusion times were varied from 1 to 12 hours when the clamps and bag were removed the abdomen was closed and the animal was allowed to recover Kidney and rectal temperatures were measured in 2 acute experiments in which a thermistor was inserted into the kidney parenchyma

Nineteen dogs were used to study the effects of cooling of a kidney

*From the Department of Surgery University of Kansas School of Medicine Supported by Research Grant #H 2363 National Heart Institute U S Public Health Service

removed with subsequent autotransplantation in the iliac fossa by the method of Murray and co-workers[3] After removal the left kidney was stored in a sterile jar at 0°C for 2 4 or 24 hours Contralateral nephrectomy was performed initially in all but the dogs with 24 hour kidney cooling in which nephrectomy was delayed for 3 to 6 weeks

Plasma creatinine concentrations urine volume and urine specific gravity were used as indices of renal function Autopsies were done on all animals except 3 which are being retained for long term survival and function studies

RESULTS

Direct measurement of kidney and rectal temperatures showed that the body temperature was maintained at 38°C during cooling of the kidney for 3 hours at 3°C

All dogs with contralateral nephrectomy total renal ischemia and kidney cooling *in situ* for periods up to 8 hours in the manner described recovered completely Animals in the 1 hour (2 dogs) 2 hour (3 dogs) 3 hour (3 dogs) and 4 hour (2 dogs) groups showed no significant elevation of plasma creatinine concentration (maximum 1 2 mg %) Diuresis for 24 hours occurred in the 4 hour group Ischemia cooling for 8 hours was followed by a transient increase of the plasma creatinine to 2 8 mg % on the following day with return to normal (1 2 mg %) accompanied by profuse diuresis All of the animals in the 12 hour *in situ* ischemia cooling group were anuric and died with uremia in 4 to 6 days Histologically the kidneys showed extensive hemorrhage and areas of infarction No histological alteration of the normal kidney architecture was observed in animals with ischemia cooling for periods up to 8 hours

Although increasing experience reduced the incidence a high proportion of the animals with autotransplantation of a removed and cooled kidney developed vascular thrombosis One dog with 1 hour cooling of a removed kidney autotransplantation and contralateral nephrectomy recovered completely with slight elevation of the plasma creatinine (2 2 mg %) and diuresis for 24 hours and is normal 1 year later The other dog used for a similar procedure developed immediate vascular thrombosis and died in uremia Of 16 dogs with 24 hr cooling of a removed kidney and autotransplantation ten developed vascular thromboses and all died with anuria and uremia after removal of the contralateral kidney 3 to 6 weeks later One animal in this group had an essentially normal appearing transplanted kidney at autopsy six weeks later with evidence of recent vascular thrombosis Direct biopsy of this kidney had been done earlier at the time of contralateral nephrectomy and showed essentially normal morphology

DISCUSSION

The observation[1] *that local kidney cooling to 20 to 25°C* will protect against renal ischemia has been confirmed and extended The observation that longer periods of ischemia are tolerated at a temperature slightly above freezing suggests a direct relationship Although none of the animals with local kidney cooling to 0° to 3°C for over 8 hours survived after removal of the contralateral kidney the finding of an essentially normal kidney histologically in one animal 6 weeks after removal cooling to 0°C

for 24 hr, and autotransplantation, suggests that it may be possible to prolong this ischemia cooling period further. Further investigations are being continued

Studies by Oliver[4] indicate that the renal tubules sustain the major damage in the ischemia kidney and steps in the repair process of this type of renal damage involve restoration of morphologic and functional integrity of the tubular units Transient diuresis, occurring in our animals with ischemia-cooling for 1 to 8 hours, is interpreted as evidence of tubular damage, and anuria accompanying longer ischemia cooling periods suggests glomerular damage as well

Surgery involving the management of aortic aneurysms, in which renal vascular occlusion may become necessary during the procedure, is a possible application of this technique. Its applicability during an operative procedure should be feasible and may afford greater protection to the kidney than general hypothermia at the temperatures of about 25°C. commonly employed.

SUMMARY

The protective effect of local kidney cooling to 0° to 3°C. for periods up to 24 hours was evaluated experimentally *in situ* and by nephrectomy with cooling of the removed kidney and autotransplantation with contralateral nephrectomy Survival of all animals and restoration of normal kidney function followed ischemia cooling for periods up to 8 hours

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ARTIFICIAL BLADDER IN MAN FROM SEGMENT OF STOMACH*

EDWIN S SINAIKO

It was previously determined in the dog[1 2 3] that an isolated gastric pouch of the Heidenhain type served well as an artificial urinary bladder. The stomach is not an absorbing organ When it is used as an artificial urinary

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bladder hyperchloremic acidosis and azotemia do not occur There is no hypokalemia because very little potassium is lost A partially continent bladder substitute and a continent urinary bladder would be physiologically feasible The contact of urine with gastric mucosa, especially antrum did not stimulate through the gastrin mechanism acid secretion in the main stomach There was no obvious damage to the ureters and kidneys from possible regurgitation of acid from the stomach In this substitute bladder the gastric acidity and lysozyme provides a bacteriostatic and bacteriocidal protective medium which is desirable in preventing serious retrograde infection in the kidneys Stomach tissue beyond the needs of the animal is available for use as an artificial urinary bladder The length of the ureters is more than enough to reach the pouch easily Cystoscopic studies of the artificial urinary bladder and retrograde studies of the kidneys could be performed easily Loss of HCl in the gastric juice secreted by the pouch did not produce hypochloremia

METHOD AND RESULTS

Having proved these contentions, and having perfected the surgical technique and approach in the dog [2] an operation to produce an artificial urinary bladder from an isolated gastric pouch was performed upon a patient, on June 30, 1956, with the assistance of Dr Theodore Burkholder and Dr Joseph Kovacs

Case report —Mrs M B a 38 year old white housewife was admitted to the Woodlawn Hospital on June 24 1956 with an extensive transitional cell carcinoma of the urinary bladder which on previous exploration had been found to be unresect able Radon seeds had been implanted into the tumor There was metastatic invasion of the regional lymphatics obstruction of both ureteral orifices and hemorrhage and anemia had occurred The patient was uremic For 5 months previous to admission the patient had been unable to void and an indwelling catheter drained urine mixed with blood

The preoperative intravenous pyelogram at 60 minutes (Fig 1) showed marked bilateral enlargement of the kidneys with gross reduction in their capacity to concen



Fig 1 Preoperative intravenous pyelogram

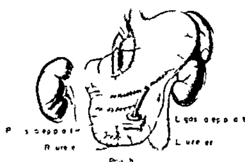


Fig 2 The suture line shows the area of resection of the greater curvature of the stomach the Heidenhain pouch was utilized as an artificial urinary bladder The kidneys are shown in their relative positions with the ureters transected at their lowermost level their distal ends tied off with silk and the proximal ureteral ends implanted into the wall of the gastric pouch and emptying into it A mucous membrane lined fistula of the gastric pouch has been brought out through the skin incision and a mushroom catheter conveys urine into a plastic bag

trate and excrete diodrast over a one hour interval and with faint demonstration of spectacular hydronephrosis

The patient was given repeated whole blood transfusions which raised the red blood cell count to a satisfactory level The operation was performed on June 30, 1956 (Fig 2)

Postoperative intravenous pyelography 7/24/56 (Fig 3) showed a reduction in the degree of bilateral renal enlargement and in the degree of bilateral hydronephrosis although marked hydronephrosis has persisted There was an apparent increase in the rate and concentration of renal excretion of 70% sodium urokon as measured by the intravenous pyelogram

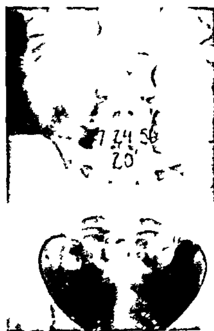


Fig 3 Intravenous pyelogram 7 24 56 postoperatively

Follow up An excretory intravenous pyelogram taken 6½ months following surgery with 60 cc of dilute diodrast injected into the artificial urinary bladder through the mushroom catheter (Fig 4) showed a rather centrally located crescent shaped artificial pouch and dilated right ureter which had filled retrograde as shown by increased density of the right renal pelvis and calyx system The left ureter had not filled by retrograde flow from the pouch

The patient had gained 8 pounds since surgery and there was evidence of normal motor function of the stomach

Cystoscopic studies of the artificial urinary bladder 6½ months following the operation showed [illegible] orifices were seen with a slight gaping on the righ[illegible] to be good on the right and fair on the left [illegible] is time showed asymptomatic aerobacter aerogenes probably due to chronic catheterization The pouch urine showed 2 to 3 WPC and one plus albumin otherwise it was normal

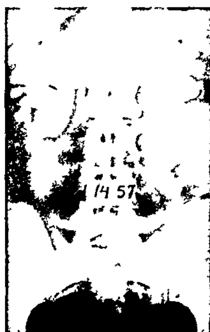


Fig 4 Intravenous pyelogram 1 4 57 6½ months postoperatively

Laboratory Studies

	PREOPERATIVELY	POSTOPERATIVELY 1 WEEK	2 WEEKS	6 MONTHS
NPN — mg %	62	84	38	38
Creat — mg %	3	1 4	1.2	1
Na — mEq	150	141	128	147
K — mEq	5 1	3	3 5	4 6
Cl — mEq	115	98	99	107
CO_2			56	70

SUMMARY

A gastric pouch has been utilized successfully as an artificial urinary bladder in a young woman with cancer of the bladder

Six and one half months following surgery there has been no evidence of severe ascending infection

There has been evidence of recovery in configuration as well as in functional ability of the kidneys

Normal function of the resected stomach has been demonstrated

Following surgery the urine returned to normal except for an asymptomatic culture of aerobacter aerogenes

When stimulated with histamine the pouch secreted hydrochloric acid

Cystoscopy revealed the mucosa of the artificial bladder to be essentially normal with open ureteral stomata

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URETEROILEOSIGMOIDOSTOMY II A REPORT OF FIVE CASES*

PERRY B HUDSON

In seeking a more satisfactory means of urinary diversion in surgical situations which demand removal of the urinary bladder, surgeons have been faced with the problems of impaired renal function as a sequel to the diversionary operations employed In a previous communication,[1] the ideal urinary transplantation operation has been described as having the following features (a) a low surgical mortality rate, (b) a convenient means of urine disposal in the postoperative period, (c) absence or low incidence of both ureteral obstruction and ascending pyelonephritis, (d) absence of electrolyte imbalance (e g hyperchloremic acidosis with or with out azotemia) (e) no features which would require compromise of the extent of radical surgery for malignant disease in order to make possible the transplantation procedure itself

In selecting ureteroileosigmoidostomy for trial in patients, attention has been given to the theoretical basis for the operation as it was used originally in dogs Interposition of a segment of terminal ileum between the ureters and the sigmoid colon has been designed to create a "physiological valve" to relieve gas and hydrostatic pressure which would otherwise be exerted upon the ureter from the large bowel, to provide a negative pressure at the end of the ureter, to act, at least in part, as a filter for bacteria between the ureter and the bowel, and to provide, through ileal peristalsis, transport of the urine into the large bowel Because of the slow rate of rhythmic segmentation peristalsis of the terminal ileum, because this portion of the intestine is less irritable than any other part of the small bowel, and because of the low resting tone and essentially unidirectional peristaltic movement, the terminal ileum has been chosen to act as a valve between the urinary and fecal passages

After successful one stage operations in dogs, the technique of uretero-ileosigmoidostomy has been employed in 5 patients The technical lessons which have been learned in the dog operations, such as placement of the

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ureters at a point not more than 1 cm from the closed end of the ileum have been incorporated into the technique used for humans

Technique of Ureteroileosigmoidostomy. The operation is performed after sterilization of the bowel by neomycin, aureomycin and cleansing enemas during the 48 hours preceding surgery

The cecum is identified through an anterior abdominal incision and the blood supply to an ileal segment within 10 cm of the cecum is isolated The ileum is transected in such a way that 15 to 25 cm of ileum will be included in the segment which is excluded from continuity with the rest of the small bowel A primary end to end ileoileostomy by a double row of interrupted fine silk sutures is employed to re establish bowel continuity The proximal end of the isolated ileal segment is closed by an inner, running, inverting suture of fine chromic catgut and an outer layer of fine interrupted Lembert silk sutures Next an end to side ureteroileostomy is performed in two layers according to the technique of Cordonnier[2] These anastomoses between the ureters and the ileum are placed within 1 cm of the line of closure at the proximal end of the isolated ileal segment Finally, an end to side ileosigmoidostomy is performed, using the two-layer type of catgut and silk closure In this way, no nonabsorbable suture material is left in contact with urine The appendix is removed and the abdominal wound is closed

Ureteroileosigmoidostomy has been employed both in conjunction with radical excisional surgical operations and separately for palliative purposes without a concomitant extirpative operation The 5 patients in whom *this procedure has been employed are as follows*

Case 1 Patient A R (F D H #2764) a 64 year old man was previously treated by a variety of surgical operations for urinary incontinence and intractable stricture of the urethra The ureteroileosigmoidostomy was performed as a single elective surgical procedure During the postoperative period the patient developed paralytic ileus which was controlled by conservative measures including Miller Abbott intubation The urinary output as collected from the rectal tube was exceedingly good and there was a noticeable lack of alteration in the electrolyte pattern of the blood This patient died of a pulmonary embolus two weeks after operation this event permitted the rare observation of all of the anastomotic connections as well as the upper tract and bowel at autopsy performed two weeks following the operation There were no unusual or abnormal findings in the kidneys renal pelves ureters the ileal segment of bowel or in any of the anastomotic connections.

Case 2 Patient A M (P H Unit #30 67 05) a 46 year old man was previously treated for bladder tumor by two transurethral resections and two open surgical operations the last of which was partial cystectomy The ureteroileosigmoidostomy was performed at the conclusion of radical cystectomy for undifferentiated recurrent carcinoma of the bladder The electrolyte balance showed the same lack of alteration as noted in Case 1 The output of collected urine in the first 24 postoperative hours was 1 500 cc, and exceeded 2 000 cc in every 24 hour period following that The patient is alive and clinically well

Case 3 Patient B R (F D H #6433) a 32 year old woman who had been treated by a radical hysterectomy operation with bilateral pelvic lymph node resection 8½ months prior to the development of a bilateral hydroureteronephrosis This patient's original diagnosis was carcinoma of the cervix secondary carcinoma was found in the left parametrium at the time of surgical pathological examination following radical hysterectomy In addition the ureterosacral ligament and right common iliac lymph nodes contained tumor During the 8 months preceding ureteroileosigmoidostomy she developed first right hydronephrosis and second a nonfunctioning (by intravenous urography) left kidney Ureteroileosigmoidostomy was performed and the patient had no deviation from normal values for serum sodium potassium chloride carbon

dioxide or nonprotein nitrogen She made an uneventful recovery, and pyelography on the 18th postoperative day showed some return of function of the left kidney, as demonstrated by the appearance of iodide in the 15 and 25 minute postinjection X rays This patient is of additional interest in that slightly more than one third of the lower ureter on each side was not thought suitable for use in the urinary diversion operation Consequently a very high transection of each ureter was made before the severed proximal end was anastomosed to the closed end of the ileal segment This patient continues to be an outpatient without demonstrable progressive damage to renal function There has been a striking gain in weight and increase in her sense of well being

Case 4 Patient J R (F D H #8339) a 65 year old man was admitted with a chief complaint of hematuria and a history of previous transurethral fulguration of the bladder Transurethral biopsy of the bladder was performed when a pathological diagnosis was made of transitional cell carcinoma of the bladder invasive After a course of radiotherapy as an out patient he was re admitted because of dysuria hematuria and spasms of the bladder During hospitalization the bleeding from the bladder could not be controlled by conservative means and his urinary symptoms were intractable Ureteroileosigmoidostomy was performed as a palliative procedure Urine appeared on the first postoperative day and continued in good amount for 10 days. After a series of complications including acute parotitis treated by X radiation the patient s course was downhill despite stabilized electrolytes and good urinary output He rapidly developed terminal cachexia associated with advanced malignancy and expired 6 weeks postoperatively Preliminary autopsy findings revealed patent anasto moses with both upper urinary tracts free of fecal material Clear urine only was found in the ileal segment

Case 5 Patient T McG (F D H #7051) a 67 year-old male who developed urinary incontinence and retention due to urethral and urethrovesical strictures following secondary retropubic radical prostatectomy for carcinoma of the prostate Failure to respond to conservative management over a period of 7 months prompted performance of ureteroileosigmoidostomy Intraperitoneal urinary extravasation was noted 7 days postoperatively and bilateral nephrostomy was performed The patient s course for the next 6 weeks was not remarkable Pyelograms revealed the left ureteroileosigmoid ostomy to be patent and functioning but stricture of the right anastomosis was apparent and revision of the anastomosis was carried out It was noted at this time that the intraperitoneal reaction was remarkably minimal and re anastomosis was performed with ease Following this procedure the patient s urinary output was excellent and the electrolyte pattern which showed a slight tendency toward hyper chloremic acidosis was easily controlled with oral sodium bicarbonate Convalescence was uneventful

DISCUSSION

Several things have been noteworthy in the use of ureteroileosigmoidos tomy in the first 5 patients There has been a prompt appearance of urine in the large bowel, usually this occurs as the final anastomosis between the distal end of the ileal segment and the sigmoid is being completed There has been an unusually high volume of urine collected even during the first 24 hours following the operation There has been observed no difficult problem in electrolytic balance, and no tendency toward azotemia or acidosis In the autopsies, on patients and on dogs, in every instance there has been only clear urine found in the ileal segment, in no instance has fecal contamination been grossly evident This is considered to be indicative of the fact that the ileal segment does act, at least partially, as a valve between the urinary and fecal passages

SUMMARY

1 The theoretical basis upon which the urinary diversion operation, ureteroileosigmoidostomy, is based is described briefly

Table 1. Urographic Findings Following the Ileal Bladder and Ureteroileocystostomy

DOG #	UROGRAMS POST ILEAL BLADDER	UROGRAMS POST URETEROILEOCYSTOSTOMY	FILLING AT CYSTOGRAPHIC STUDY	TOTAL TIME FOLLOWED (WEEKS)
1	+	N	0	30
2	N	*	*	17*
3	N	N	Ileum	24
4	++	N	0	24
5	+	N	Ileum Left Ureter	20
6	+	N	0	20
7	+	*	*	6*
8	N	N	Ileum Right Ureter	16
9	N	N	Ileum Left Ureter	12
10	+	N	Ileum	11

N = Normal
\+ = Moderate Hydronephrosis
++ = Marked Hydronephrosis
* = Dead

Table 2 Blood Chemistries†

	BLOOD UREA NITROGEN mg %	CREATININE mg %	CARBON DIOXIDE CONTENT mEq /L	CHLORIDE mEq /L	SODIUM mEq /L	POTASSIUM mEq /L
Post Ileal	38	1 1	24	112	148	4.5
Bladder	23 55	0 9 1.3	21 25	104 118	140 152	3 9 5 0
Post Uretero	21	1 0	23	112	146	4 9
ileocystostomy	15 29	0 8 1 1	21 26	107 117	143 151	4 6 5.3
Control Values	18	0 9	23	111	148	4 8
	11 26	0.8 1 0	20 27	108 113	145 150	4 7 5 1

† The average and range of the blood chemistry determinations are given The Post Ileal Bladder values are from the dogs subsequently given an ureteroileocystostomy The normal values for blood urea nitrogen and serum creatinine were derived from ten healthy dogs and from four dogs for the serum electrolytes

Retrograde cystographic study revealed a valve like action present at either the ileocystostomy or ureteroileostomy in many animals

The dogs with some radiographic evidence of hydronephrosis after the creation of the ileal bladder had a moderate elevation of the blood urea nitrogen following that procedure There was however, no hyperchloremic acidosis evident After the ureteroileocystostomy, blood chemistries were considered to be normal except for a slight elevation of the blood urea nitrogen and serum creatinine observed in 2 animals All animals were healthy in all respects and either maintaining or gaining weight

DISCUSSION

The results of the presently short follow up in this series would indicate the safety with which the ileal bladder may be used as only a temporary external diversion of the urine stream All complications which occurred in this series were technical in origin and none could be directly placed *per se* on the presence of the ileal segment The importance of technically perfect ureteroileal and ileal cystic anastomosis is emphasized

Bladder function remained excellent in these animals after the uretero-ileocystostomy, although some mucous was present in the urine of all dogs As seen from the serial excretory urographic studies, there appeared to be no deleterious hydrostatic back pressure effect on the ileal segment, ureters or kidney The presence of the short ileal segments used did not provide sufficient surface area for electrolyte reabsorption

The final anatomical state was no different than that proven in the experimental studies of Davids and Lesnick [3] When innervation of the bladder is intact, the possibility for the use of an ileal segment in the salvage of bladder continence where it may otherwise be lost, such as with congenital exstrophy of the bladder and intractable vesico-vaginal fistula is evident

A long term study of these dogs is in progress

CONCLUSIONS

Temporary external diversion of the urinary flow to an ileal segment was performed in 14 dogs In 10 dogs the normal urine flow tract was later reconstituted by a ureteroileocystostomy Blood electrolyte and excretory urographic studies revealed no marked abnormalities in the 8 short term follow ups

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CYSTOPLASTY TO INCREASE BLADDER CAPACITY—I*

Experimental Use of Isolated Patches and Loops of Large and Small Bowel to Increase Urinary Bladder Capacity in Animals

CHESTER C WINTER, JOHN BRIGGS, AND WILLARD E GOODWIN

Severe contracture of the urinary bladder due to inflammatory lesions, neurological disease, or chemical injury may present a formidable problem to the urologist A patient's distress may force the surgeon to think in terms of removal of the bladder and diversion of the urine An alternate approach is substitution of a new bladder or plastic enlargement of the contracted urinary reservoir, using isolated segments of intestine[1] Technique of the latter surgical treatment has varied widely and lack of uniform results has stimulated considerable experimentation concerning anatomy and physiology of the new "intestinal' urinary bladder The variable absorption of electrolytes from the ' new ' bladder has been the subject of intense study[2,4,5]

The present animal studies were initiated in 1953 and presented in part as a preliminary report to the Clinical Society of Genito-Urinary Surgeons, February 1954 Several experimental methods of increasing bladder capacity were studied in the dog

METHOD

Nine average sized dogs of both sexes were used In 4 animals, intestinal 'patches' were added to the normal bladder dome which had been incised In 2 of these, the intestinal mucosa was removed from the 'patches" In

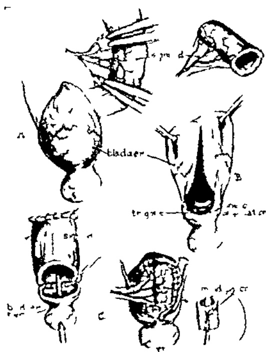


Fig 1 Operative steps in intestinal cystoplasty (loop and patch)

*From the Department of Surgery Division of Urology, University of California Medical Center Los Angeles

the remaining 5 animals, three-fourths of the urinary bladder was amputated and substitute intestinal bladders were fashioned and attached to the trigone as patches and in one experiment as a diverticulum. The intestinal mucosa was stripped from 3 of these new bladder transplants (Fig. 1).

Five day preoperative bowel preparation consisted of daily sulfathaladine and cascara. Cleansing enemas were given on the 2 days prior to operation. Pentobarbital anesthesia was employed.

Intestinal-vesical anastomosis was performed with multiple 4-0 chromic catgut continuous sutures. Intestinal continuity was restored with a single row of interrupted 4-0 silk sutures using a closed technique.

RESULTS

As a preliminary study in the first animal, a 5 cm. segment of sigmoid colon was isolated, opened on its antimesenteric border, and then applied as a patch to the opened dome of the bladder for enlargement. End-to-end sigmoid anastomosis restored bowel continuity. Postoperative function of this bladder was good and residual urine small. Considerable free mucus was found in the bladder at the time of autopsy, one month postoperatively.

In a second animal, all of the urinary bladder above a cone containing the trigone and ureters was amputated. An isolated 7 cm. segment of sigmoid colon was opened on its antimesenteric border and sutured as a patch to the remaining cone of the original bladder. There was no residual urine 6 weeks postoperatively. Bladder capacity was 180 ml. eight weeks postoperatively.

In dog 3 the same procedure was employed, and in addition the sigmoid mucosa was stripped from the muscularis before an anastomosis of the muscular-serosal patch of bowel to the cone of original bladder. The bladder capacity 5 weeks postoperatively was 50 ml. with a 3 ml. residual. The urine was yellow and slightly turbid. Microscopic examination of the centrifuged urine revealed many bacteria and white blood cells. An atonic bladder was demonstrated cystometrically at the end of the third postoperative month without the use of anesthesia. The dog was then sacrificed. Microscopic examination of the "patch" revealed chronic inflammatory changes and a new transitional cell epithelium appeared to have formed over the muscular layer of sigmoid.

Dog 4 had the same operation as the third. Bladder capacity at 2 months was 55 ml. and residual was 8 ml. Many white and a few red blood cells and bacteria were seen per high power field in the centrifuged urine.

In animal 5 the bladder was amputated just above the trigone level. A 6 cm. segment of sigmoid was isolated and the proximal end closed. The distal end was anastomosed to the bladder as an isoperistaltic diverticulum. This dog died in a few days from sepsis.

In animals 6, 7 and 8, the bladder was not amputated. This dome was incised vertically and an isolated 6 cm. segment of distal ileum was used as a patch applied to the opened bladder. The ileal mucosa was stripped prior to anastomosis. One dog died of a complete small bowel obstruction at the site of end-to-end ileo-ileostomy 2 weeks postoperatively. The new bladder appeared intact. The two remaining dogs were sacrificed at the end of the second and third postoperative months, respectively.

Microscopic examination of the patches revealed new transitional epithelium with tiny islands of intestinal mucosa

In dog 9 the bladder was amputated above the trigone and 2 isolated 10 cm segments of distal ileum were opened and sutured together side by side to make a large rectangular patch This patch with its double blood supply was then anastomosed to the remaining bladder This dog also died shortly postoperatively The bladder was filled with thick mucus

DISCUSSION

The sigmoid colon and distal ileum because of their proximity mobility and viability lend themselves readily to transplantation to the urinary bladder The artificial bladders thus made remain viable form satisfactory urinary reservoirs in short term followup studies and give no problems of urinary residual or incontinence In dogs 3 4 5 7 and 8 the mucosa was stripped from the bowel prior to cystoplasty In the 3 animals that lived the regenerated epithelium was of the transitional rather than the intestinal glandular type (Figs 2A and B) This finding is of considerable importance If it occurs consistently two disadvantages of the use of bowel for enlarging the bladder would be overcome 1) the absorption of urinary electrolytes through bowel mucosa and 2) the formation of mucus by intestinal glands Shoemaker[3] has used reversed seromuscular bowel grafts after removing the mucosa in order to achieve the same goal

Although the above mentioned disadvantages are prevented an interstitial cystitis occurred in one of our dogs This may result in smaller bladder capacity than is obtainable with full thickness bowel grafts

The use of a patch rather than a closed segment of bowel may be advantageous The ability of this rectangular transplant to contract and function as a bladder does not appear to be lost and the shape of the new bladder is more consistent with the normal vesical contour

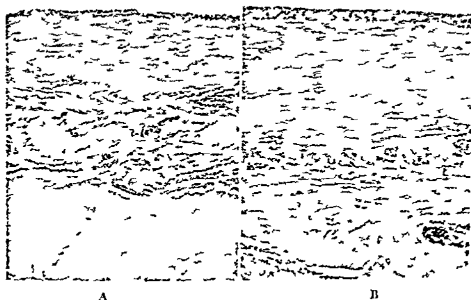


Fig 2A and B Transitional epithelium has formed over the denuded muscular layer of bowel

The postoperative cystogram in one animal revealed unilateral ureteral reflux This may be a complication of intestinal cystoplasty due either to increased bladder tone and pressure or to impairment of nerve or blood supply to the ureteral vesical junction near the suture line

The surgical technique of intestinal cystoplasty in both dog and human is not difficult nor need it be a lengthy operation In the human the urinary bladder is less accessible in the trigone area Diseases for which cystoplasty may be performed could make the procedure more difficult and time consuming Nevertheless intestinal cystoplasty is a practical surgical procedure worthy of continued experimental study as well as clinical application for the purpose of enlarging the contracted human urinary bladder

A clinical report on technique and results of enlargement of the bladder by a patch of ileum in 8 patients and a patch of sigmoid colon in 1 patient is to be the subject of another communication

SUMMARY AND CONCLUSIONS

Enlargement of the urinary bladder by use of an ileal or sigmoid patch or unopened segment was performed in 9 dogs The procedure is feasible from the standpoint of surgical technique bladder function capacity and urinary residual Ureteral reflux may occur when only the trigone of the original bladder remains

The mucosa was removed from 5 transplants in order to prevent the absorption of urinary electrolytes and the formation of mucus In all instances the regenerated epithelium was transitional (bladder) rather than columnar mucus secreting (bowel) Long periods of observation will be necessary to evaluate the degree of vesical contracture due to the interstitial cystitis that may occur following the latter procedure

Intestinal cystoplasty is applicable to humans for the purpose of increasing bladder capacity It may also have its most important use in certain types of neuromuscular bladder disease where the normally innervated ileum is used as a substitute for the diseased detrusor of the bladder

Several successful clinical results are the subject of another communication

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EFFECT OF ADRENOLYTIC, ADRENERGIC, ANTICHOLINERGIC, CHOLINERGIC AND ANTIHISTAMINIC DRUGS ON MICTURITION*

JACK LAPIDES, NORMAN B HODGSON, AND ROBERT E BOYD

Modern concepts of bladder physiology indicate that only the cholinergic and anticholinergic drugs should affect micturition However, numerous reports in the literature describe difficulties in urination produced by anti histaminic agents and by drugs stimulating or paralyzing the sympathetic nervous system In view of the discrepancy it was decided to investigate the reaction of the urinary apparatus to drugs possessing adrenergic, adrenolytic, cholinergic, anticholinergic, and antihistaminic properties

METHOD

In order to obtain objective information, paraplegics with reflex neuro genic bladders were employed as the initial subjects These patients have spinal cord lesions completely interrupting sensory and corticoregulatory tracts at a level higher than the sacral spinal cord Reflex bladders are characterized by lack of sensation, and lack of cortical control resulting in uncontrolled or uninhibited contractions of the detrusor When the reflex neurogenic bladder is filled with urine or fluid, proprioceptive endings are stretched and sensory impulses are initiated, they travel along afferent fibers to the spinal cord where they impinge upon the motor neurones of the bladder and cause them to discharge The motor impulses then travel over parasympathetic fibers, ganglia and neuromuscular junction to stim ulate contraction of the vesical musculature Within limits, the investigator can stimulate bladder contractions at will in these patients by filling the bladder with a certain volume of fluid at a set pressure and rate of flow, and these contractions can be reproduced during several runs provided an adequate rest period is allowed between fills The paraplegic is the most physiological subject for these studies because anesthesia is not needed, the bladder with its lower reflex arc is intact and varying cortical influences are eliminated

Cystometry[1] was used in evaluating the bladder reactions to the drugs The cystometric apparatus was connected to an inlying urethral catheter *in some patients and to a suprapubic cystostomy tube in others* At least 2 control cystometric examinations were performed in each patient prior to the administration of a drug Patients with marked variability in their control cystometrographs were eliminated as subjects All drugs were administered parenterally and in dosages usually higher than those recom mended for routine use After an appropriate time interval, usually 10 to 20 minutes after injection, several cystometrographs were again obtained Some patients agreed to have all of the drugs given to them while others permitted the administration of only 2 or 3 drugs Each drug was tested in 10 patients

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Table 1 Types of Compounds Utilized, their Dosages and Routes of Administration

COMPOUND	TYPE	DOSAGE	ROUTE OF INJECTION
Ephedrine	Adrenergic	0.075 gm	IV
Regitine	Adrenolytic	0.010 gm	IV
Urecholine	Cholinergic	0.007 0.0010 gm	Subcutaneous
Neostigmine	Cholinergic	0.004 gm	Subcutaneous
Banthine	Anticholinergic	0.050 0.100 gm	IV
Atropine	Anticholinergic	0.0012 gm	IV
Benadryl	Antihistaminic	0.050 0.075 gm	IV
Pyribenzamine	Antihistaminic	0.050 0.075 gm	IV

After completion of administration of the drugs to the patients with reflex neurogenic bladders the various compounds with the exception of atropine were tested in subjects with normal bladders some of these were medical students and house staff The subjects were advised to present themselves to the investigator when they had a strong desire to urinate At this time they were given an injection of one of the drugs and instructed to attempt to empty their bladders within 10 to 20 minutes After visiting the urinal they returned to the investigator and described their experiences Each drug was given to 10 normal subjects

RESULTS

The adrenergic adrenolytic and antihistaminic agents (Ephedrine Regitine Pyribenzamine and Benadryl) demonstrated no effect on the cystometrographs of the reflex neurogenic bladders or the voiding ability of the normal subjects despite relatively enormous doses of the drugs

The anticholinergic drugs atropine and banthine, depressed or abolished the uninhibited contractions of the reflex neurogenic bladders, basic detrusor tonicity was not affected Figure 1 depicts a typical reaction to atropine The responses of the normal subjects to 50 mg of banthine intravenously included decreased urge to void difficulty in initiating urination weak interrupted stream and feeling of incomplete emptying of the bladder

The cholinergic drugs urecholine and neostigmine, produced a change in the function of the reflex paraplegic bladders if they were given in a sufficient dosage In the preliminary testing period it was observed that

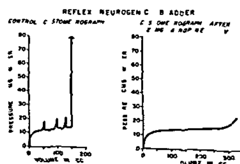


Fig 1

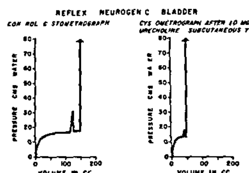


Fig 2

5 mg of urecholine or 1 5 mg of neostigmine subcutaneously had no effect on some of the reflex bladders Consequently the dose was doubled for both compounds and responses were elicited regularly The cholinergic drugs stimulate the bladder to contract at a smaller capacity and with greater force, basic vesical tonicity is not affected Figure 2 illustrates the response of a reflex neurogenic bladder to urecholine

When the normal subjects were given the cholinergic compounds the most common reaction was an increased desire to urinate in several subjects the desire was uncontrollable It is interesting to note that when the bladder is empty, urecholine does not initiate a desire to urinate Most of the patients did not notice any increased ease or force in urination

DISCUSSION

The observations made during this study demonstrate that drugs altering parasympathetic activity do affect bladder function These findings are entirely in accord with present concepts of bladder physiology which hold that the motor fibers to the bladder are parasympathetic The sympathetic nervous system plays no part in the process of micturition

Ephedrine has been found to be effective by many physicians in decreasing nocturnal enuresis in children Some ascribe its effectiveness to stimulation of the internal vesical sphincter by virtue of its adrenergic action this is pure armchair theorization and is completely contrary to all valid experimental evidence Most physicians now agree that the therapeutic value of ephedrine lies in its ability to make patients sleep less soundly and thus be more aware of a full bladder

The case reports describing urinary complications associated with the use of antihistaminic and adrenolytic drugs discuss patients receiving several medications simultaneously and having multiple complaints Critical analysis of these reports reveals that there is little or no basis for ascribing the urinary complaints to the drug implicated

When administered in a single dose none of the drugs used in this study, (including the cholinergic and anticholinergic compounds) had any influence on the inherent tonicity of the bladder muscle This property of bladder muscle is similar to the response of ureteral smooth muscle to drugs

CONCLUSIONS

1 Adrenolytic adrenergic and antihistaminic substances have no effect on urinary bladder or sphincter function

2 The anticholinergic drugs (atropine, banthine) block transmission of motor impulses to the bladder and thus tend to inhibit detrusor activity

3 The cholinergic drugs (urecholine, neostigmine) accentuate the effect of the motor impulses on the detrusor and cause increased voiding contractions

4 None of the drugs tested in a single dose had any effect on inherent bladder tonicity

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EVALUATION OF A NEW SPIRAL TECHNIQUE FOR THE CORRECTION OF DEFECTS OF THE URETER*

JOEL L ALVIS, JULIAN WIENER, AND TOM D NORMAN

Surgical excision of a section of human ureter is sometimes necessary because of neoplasm, injury, or adjacent pathological changes End to-end anastomosis of the ureter is satisfactory when there is no tension on the suture line Otherwise urinary diversion or nephrectomy is required A method of satisfactorily bridging larger defects has been considered and experimental work carried out on canine ureters This was based on previous work by Davis[1] (Fig 1 A) who has shown that ureteral regeneration will occur if a strip of intact ureter is left to bridge a surgically created ureteral defect Huffman, McCorkle, and Persky[2] have shown that smooth muscle regeneration does not result when there is intubated ureterotomy without a ureteral bridge (Fig 1 B)

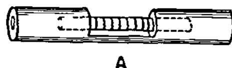
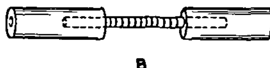


Fig 1 See accompanying text



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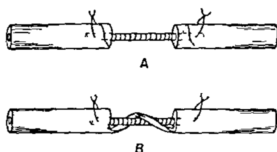


Fig 2 Diagram showing method of creating pedicle flaps to bridge defect

METHOD

This surgical procedure was carried out on mongrel dogs under nembutal anesthesia using an extraperitoneal lumbar approach A total of 22 ureters was exposed and operated upon in the following manner

After excising a 4 cm strip of ureter a ureterotomy tube was inserted into the intact ureteral ends and transfixed on each side of the defect The ureterotomy tubes consisted first of woven ureteral catheters but for technical reasons polyethylene tubing was later substituted A spiral pedicle flap was then created from each ureteral end and the two were sutured together over the tube (Figs 2 A and 2 B) Black silk sutures were left at each end of the ureteral defect for later identification A Penrose drain was then placed in the area of the anastomosis and the wound was closed in layers Intramuscular penicillin and streptomycin were given for several days postoperatively The dogs were sacrificed at varying intervals from 2 weeks to 6 months and transverse or longitudinal sections made at several levels through the defect Histologic studies utilizing hematoxylin and eosin as well as Gomori's trichrome stain were made of multiple sections through the defect and the area above and below the defect Histologic studies of the kidneys were done in 18 cases

RESULTS

In 4 of the 22 ureters there was such a marked degree of local infection that no histologic studies were made Little or no muscle regeneration was present in sections of 5 ureters which showed marked inflammatory reactions or abscesses Longitudinal sections were made on 5 ureters and smooth muscle was seen throughout most of the length of these but the results were considered nonconclusive Of the remaining 8 ureters smooth muscle was found about the circumference of the ureter at all levels in 3 cases (Fig 3) In some of the sections through the defect the musculature



Fig 3 Transverse section through area of defect at interval of 10 weeks following surgery Smooth muscle and mucosa completely surround the lumen X40 Hematoxylin and eosin stain

was not as thick as in the normal ureter The other 5 showed incomplete regeneration in at least one of the sections As has previously been described, bone formation was occasionally noted in the periureteral scar tissue This was noted in 6 of the ureters in this experiment, one where longitudinal sections were taken and 5 where there was incomplete smooth muscle regeneration Thus, in 5 of the ureters where the smooth muscle did not completely circumscribe the lumen, bone formation was present but the significance of this is not known Mucosa completely surrounded the lumen of the defect in all sections of 7 cases, and in 6 cases regeneration was incomplete

Grossly, 15 of the 18 kidneys sectioned appeared normal All 15 of these showed mild to moderate pyelonephritis on histologic sections Of the other 3, 2 showed severe pyelonephritis with pyonephrosis, and 1 showed severe pyelonephritis with hydronephrosis In general the least altered kidneys were associated with the best ureteral repairs

DISCUSSION

The results indicate that in dogs spiral pedicle grafts can be used to span a ureteral defect with subsequent regeneration of mucosa and smooth muscle It is understood that the term "regeneration' is used rather loosely since some authors believe that this may represent smooth muscle hyper trophy or hyperplasia Recent work by Hinman and Oppenheimer[3,4] suggests that true regeneration does occur, but this has not been definitely proven

The above results in which only 3 of the ureters showed regeneration in all sections may not be impressive, but the technical difficulties in handling small canine ureters must be considered The diameter of the ureter is often only 1 mm, producing a spiral flap only 1 to 2 mm wide Despite the fact that drains were cut flush with the skin, the dogs fre quently removed these within a short time Most of the poor results were in cases where there was inadequate drainage and infection was not controlled

It is interesting to note that in the 3 ureters showing complete smooth muscle regeneration at all levels, the animals were sacrificed at 3 weeks, 5 weeks and 10 weeks after operation No conclusion can be drawn from this, but additional experiments may give more precise information concerning the time required for complete circumferential regeneration One must be cautious in assuming that the same result will occur in man, but it is known that this type of regeneration does occur in the Davis[5] type ureterotomy

SUMMARY

A method of spanning a surgically created ureteral defect in dogs has been presented Results indicate that where there is adequate drainage and infection is controlled complete ureteral regeneration may occur.

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Author Index

Subject Index